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Relocation of the Enhancer transposon in maize

by

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INTRODUCTION

Heritable variegation affecting many traits is a widespread phenomenon among plants. Because of the ease of investigation, variegation of kernel coloration in maize (Zea mays) has received the most intensive research. In recent years, phenomena associated with variegation in plants have been related to similar features in bacteria, Drosophila, yeast, and other diverse organisms.

In cases receiving intensive investigation, variegation in maize has often been shown to be caused by controlling elements, elements that are known to insert into assorted loci and thereby control gene activity. The interaction of controlling elements with the controlled allele can result in the turning on and off of genes resulting in mutations showing changes from the recessive to the dominant phenotype. This mutability is inherited as a Mendelian trait.

Controlling elements in maize appear in either a one or a two component system. There are presently four established two-element systems in maize. This study is concerned with one of these systems, the Enhancer controlling element system. The two-element system is comprised of two components, a receptor and a regulatory element. The receptor element always resides at the mutable locus and its presence usually limits gene activity. The regulatory element can reside anywhere within the genome and by a transactive function causes the release of gene activity. The timing and frequence of gene release are under precise, heritable control.

One of the unique properties of controlling elements is their ability to transpose from one location in the genome to another. The present study

is concerned with the movement of the Enhancer regulatory element from the A1 locus to a limited area of the long arm of chromosome 3. The transposition of this element was studied in an attempt to answer some questions about the process of transposition and its relation to chromosomal structure and function.

1. Is the position of the transposed regulatory element Enhancer (En) random or are there preferred regions of insertion within this chromosome region?

2. Is the transposition behavior of En's from different sources similar?

3. Do the En's located in some chromosomal regions transpose more often than those located in other regions? Are the En's inserted in some regions less likely to transpose and, thus, more stable?

4. How far does En move and is there a tendency for it to move a certain direction in relation to the centromere?

5. Does the distribution of En in relation to the A1 chromosomal site change in subsequent transpositions?

6. Is there a correlation between the chromosomal position of En and the timing and frequency of gene release?

LITERATURE REVIEW

Anthocyanin Pathway

Much of the research on maize controlling elements has been concerned with their effect on endosperm characteristics and especially on kernel coloration. A review of the genetic control of anthocyanin synthesis in maize will help to clarify some controlling element terminology.

Red and purple kernel color is seen in either the pericarp or the aleurone layer. The pericarp is the thin, outermost layer of maternal tissue surrounding the kernel. The presence of the dominant P locus on chromosome 1 is essential for pericarp coloration. The aleurone is the outermost layer of endosperm tissue directly beneath the pericarp. It is triploid, derived from the fusion of one pollen sperm cell and the two polar nuclei of the embryo sac. For color to be produced in the aleurone at least one dominant gene must be present at each of the following loci: A1, A2, C1, C2, and R. C1-I, an allele of C1 is a dominant color suppressor, irrespective of the remainder of the genotype. In addition to the main color genes there are several modifier genes such as bronze 1 (bz1), bronze 2 (bz2), red (pr), and intensifier (in) that modify the amount or form of anthocyanin produced when in the homozygous recessive state (Neuffer et al., 1968).

Using tissue complementation techniques, the sequential order of gene action of most of the color producing genes has been determined. The genes act in the following order: CI-C-C2-R-In-A1-A2 (Reddy and Coe, 1962). Reddy and Reddy (1975) have proposed a partial biosynthetic

pathway. Flavanol is produced from an unknown precursor by the action of C1, C2, and R. Flavanol is converted to flavenol by the A1 gene product. The conversion of flavenol to anthocyanin is catalyzed by the A2 gene product. When the pathway is blocked at A1, quercetin will accumulate, while leucocyanidin concentration builds up when the pathway is blocked at A2. The a1-mr and a2-mr alleles are under the control of the En system and are colorless in the absence of the En regulatory element. As expected, quercetin will build up in a1-mr/a1-mr kernels and leucocyanidin accumulates in a2-mr/a2-mr kernels in the absence of En (Reddy and Peterson, 1976). The receptor elements of the En system at these loci do suppress gene activity at the corresponding step in the biosynthetic pathway.

Controlling-element Systems

A two-unit controlling element system includes a regulatory and a receptor element. A system is identified by the specific response of a receptor element to its own regulatory element. Controlling element systems exist in either an autonomous or a two-element form. In the autonomous form the receptor element and regulatory element are both located at the controlled locus. In the two-element system the receptor element is located at the controlled locus while the regulatory element can be found at any other location in the genome. The two-element system arises from the autonomous form and the receptor element behaves like a remnant of the regulatory element that has the ability to repress gene

activity but lacks the ability to reactivate the gene (Peterson, 1960; McClintock, 1962).

Four regulator-receptor systems are identified in maize by their specific regulatory element-receptor element interaction. They include: Activator (Ac), Dissociator (Ds) (McClintock, 1945), Dotted (Dt) - al-dt (Rhoades, 1938), Enhancer (En) - Inhibitor (I) (Peterson, 1953), and Factor cuna (Fcu)-rcu (Gonella and Peterson, 1977). Modulator (Mp) is identified with Ac in its effect on Ds (Barclay and Brink, 1954) and Suppressor-Mutator (Spm) with En (Peterson, 1965a).

In the systems other than En the receptor element by its cis position at the controlled locus blocks gene activity and the regulatory element releases this inhibition by excising the receptor element. The En-I system controls more complex patterns of gene expression.

The En regulatory element's effect on a responsive allele (one with the I receptor element) is both to suppress and to activate gene activity. With some controlled loci the I element will permit a modified gene expression but at a lower level of gene activity in the absence of En. With En, gene activity will be either completely suppressed or activated. En suppression of gene activity can be seen in the al-m-1 allele (McClintock, 1965). When En is absent, the I element at the al-m-1 locus allows some gene activity and a pale coloration is seen in al-m-1 kernels. When En is present, the pale coloration is completely suppressed yielding a colorless background, but spots of full color are also seen in the aleurone tissue. The Enhancer transmits both a suppression (S) and a mutator (M) message to its receptor. The S message suppresses the pale

background color of the a1-m-1 allele and is thus responsible for the colorless background. The M message reactivates the gene and produces the full colored sectors. These special functions of the Enhancer element imply that it contains separate genes encoding these two messages (Nevers and Saedler, 1977).

The F-cu system, found in a Colombian race of maize, has been recently described by Gonella and Peterson (1977). F-cu is related to the Spf controlling element. Spf induces mutability of the R-r#2 dil allele (Singh et al., 1975), but the two regulatory elements are not identical. F-cu causes spotting on both the R-r#2 dil and r-cu alleles but the Spf effect is limited to R-r#2 dil (Gonella and Peterson, 1978). The receptor at r-cu will not respond to Spf. This is an exception to the usual specificity of receptor-regulator interaction.

There are several other examples of inherited instability in maize that appear to be due to controlling element activity such as the R-st allele (Kermicle, 1970), but the genetic control of this allele is not as clear as in the other controlling element systems.

Origin and Distribution

Controlling elements are common components of the maize genome. They are present in many geographically diverse races of maize and have often been induced by radiation in maize lines where their presence had not been previously detected (McClintock, 1945; Doerschug, 1973; Peterson, 1953).

There are several independent origins of Dt. Dt1 was originally recognized in progeny from a selfed population of Black Mexican sweet corn

(Rhoades, 1938). It causes spotting in Emerson's *al* tester, but not in other *al* sources. *Dt2* was found in a Brazilian and *Dt3* in a Peruvian race of maize (Neuffer, 1955). *Dt4* and *Dt5* arose in material that had undergone bridge-breakage fusion cycles on chromosome 9 (Doerschug, 1973).

Emerson (1914) collected "freak" ears at local and national corn expositions for his study of the variegated pericarp (P-vv) trait. *Mp*, the responsible controlling element, has been identified in maize from eight geographic areas in Canada, the U.S., and S. America (Barclay and Brink, 1954).

Ac + *Ds* were first recognized in maize lines undergoing the bridge-breakage-fusion cycle after x-irradiation to produce deletions for gene mapping (McClintock, 1945). Although *Mp* has never given rise to a two-element system at the *P-vv* locus, it is assumed to be identical to *Ac* because it triggers *Ac*-like *Ds*-induced mutations (Barclay and Brink, 1954).

En was first detected in progeny of maize kernels irradiated at the Bikini A-bomb test site (Peterson, 1953). It was originally associated with the *pg-m* allele and caused mutability in maize leaves and stems from pale green to full green. *Spm*, which is identical in activity to *En*, arose spontaneously in McClintock's maize lines during an experiment designed to detect new mutable alleles under *Ac* control at the *A1* locus (McClintock, 1953). The widespread occurrence of *En* has been verified by its presence in five out of twenty-three tested lines of maize from Mexico, Colombia, Bolivia, and Venezuela (Gonella and Peterson, 1975).

The widespread occurrence of controlling elements in geographically and genetically diverse populations underlines their ancient association with maize. Their frequent appearance following radiation treatments in lines where they had not been previously detected led to speculations that they could be present in genetically inert heterochromatin (Brink, 1964; McClintock, 1951). In many cases, Ds associated chromosomal rearrangements involved heterochromatic regions. It was thought that controlling element suppressions could be caused by a "heterochromatization" of the mutable allele. This effect could diffuse along the chromosome causing the spreading effect noted in Ds lines (McClintock, 1955) and in mutable allele systems in Drosophila (Green, 1967). However, as more information became available on gene control in prokaryotes other possible mechanisms of controlling element activity were suggested. The similarities to the operon systems (McClintock, 1962) and later to transposable elements in bacteria (McClintock, 1963) became apparent.

The transposable elements in bacteria control gene activity by inserting and excising from the host DNA and controlling elements in maize seem to behave in the same way.

Evidence for Insertion

Controlling element mediated mutability is an inherent property of the affected allele and all the evidence indicates that controlling elements are actually inserted into the maize DNA at the controlled locus. This is substantiated by several observations.

Controlling elements are closely associated with the maize chromosome. They are inherited as simple Mendelian traits (Fincham and Sastry, 1974) and both meiotic and mitotic transmission is normal. Linkage data invariably indicate a close association between the autonomous controlling element and the controlled allele. In the two-element system the receptor element is always linked closely to the mutable locus. Out of 791,096 kernels no cross-overs were found between A1 and the receptor dt-factor (Neuffer, 1965).

Some of the most convincing evidence for controlling element insertion into the maize DNA is provided by interallelic mapping of the waxy locus (Nelson, 1968). Three receptor elements were mapped against a number of spontaneous waxy mutants. They were located at three separate sites within the waxy locus where they behaved like simple point mutations. Interallelic mapping within this locus is facilitated by the ability to follow recombinant events in the pollen. Wx pollen grains stain deep blue in an I2-kI solution while wx grains are lightly stained. Since large numbers of pollen grains can be easily screened,interallelic recombination can be detected. The mutable alleles wx-m-1 and wx-m-6 responding to Spm were mapped against a number of deletions and point mutations. The responsive alleles are completely stable in the absence of their respective elements. They apparently caused no cross-over interference and recombination was normal.

Evidence for Excision

Activation of alleles responding to controlling elements is due to an excision event. Although the process is sometimes referred to as

mutation, the mechanism is quite different. In controlling element systems the change from null or low expression to full expression can be seen in hundreds of cells per kernel but in standard genes, reversions of null mutants to a normally functioning state occurs at a very low rate. While instability of an allele under controlling element influence is normally inherited, most mutations occur once and cause a permanent change in gene activity. It is evident that the instability seen in mutable alleles is due to a process of gene deactivation and activation rather than to a permanent change in the structural gene itself.

Evidence that the change in gene activity is caused by excision of the controlling element is found in the Ds-Ac system (McClintock, 1951). The presence of Ds was first detected in x-irradiated lines because it produced breaks which were always found at a definable point along the chromosome. Loss of the non-centric portion of the chromosome revealed recessive genes in the paired segment and resulted in a specific pattern of sectoring in the kernel. It was later found that an independent factor, Ac, triggered the breaks at the Ds location (McClintock, 1946). When Ac was absent from the genotype Ds was stable. Eventually, states of Ds arose that would cause mutability in response to Ac, but without apparent chromosome breaks (McClintock, 1953). In these states, as in most of the controlling element systems, excision appears to be precise and is followed by precise repair of the DNA strand.

The timing and frequency are under the precise control of the regulatory element and in its absence the receptor element is stable.

Although radiation can induce chromosomal breaks, radiation cannot mimic the effect of the regulatory element on a receptive allele (Stadler, 1944). Radiation will cause mutations of a1-m to a1 but produces no reversions of a1-m to A1 (Neuffer, 1966) although regulatory elements always produce gene activations on receptive alleles. The regulatory elements must be able to cause an exact excision of the receptor element. The differential effect of temperature on the rate of sectoring in different systems suggests an enzymatic reaction (Peterson, 1958).

Further evidence supporting the concept of controlling element insertion and excision is found in their ability to transpose from one position in the maize genome to another.

Transposition

The ability to transpose is one of the unique features of the controlling elements. Standard genes occupy a set position in the genome and linkage relations remain fixed except for rare chromosomal rearrangements. Controlling elements can occupy many chromosomal sites and change positions in the genome with or without apparent chromosome breakage.

Controlling element transposition was first observed by McClintock (1951) in the Ac system. The original position of Ds was just proximal to Wx on chromosome 9. In crosses between C-I Sh Bz Wx Ds females and Ac, C sh bz wx males, breaks could be followed at the Ds locations. This was followed by the loss of the non-centric fragment uncovering sectors of C sh bz wx phenotypes. Some exceptional kernels were found with colored sectors, not accompanied by sh, bz or wx sectoring suggesting that the Ds

position had moved to the C locus and the C-I allele alone was lost. Cytological examination confirmed a change in the Ds breakpoint in the exceptional material to the C locus. Similar evidence for Ds transposition to the Bz1 and the Sh1 loci has been described.

In many cases, Ds transpositions involved chromosomal rearrangements. This led McClintock (1950) to propose that during transposition Ds became "sticky" and adhered to various sites within the maize genome. Separation of chromosomes during mitosis resulted in breaks in the chromosomes.

In the other controlling element systems no chromosomal breakage is induced but transposition can be detected by following changes in linkage relations. The first recognized Dt element was located on chromosome 9, seven map units from yg-2 (Rhoades, 1945). Two Dt elements induced by chromosomal breakage were found at two separate sites, one at 33 map units from yg-2 and the other on 7L (Doerschug, 1973). McClintock's Spm was traced to two positions on chromosome 6, two on chromosome 5, two on chromosome 9, and to several other positions (McClintock, 1957). In most cases, loss of the controlling element at the original position and reappearance at a new position could be verified.

Transposition of the controlling element away from a receptive allele results in loss of mutability at that site. With specific alleles of controlling element systems the loss of the controlling element will give rise to stable germinal derivatives. In many cases, transposition of the controlling element to a new position can be confirmed.

McClintock (1956) found that in 16 out of the 24 cases of stable recessive derivatives from the bz1-m-4 allele Ac was removed from the locus. In 7 of the 16 cases, it was detected at a new location. The rate of transposition was probably underestimated, however. In at least some of the apparent Ac losses the transposed Ac may have been removed by segregation. Peterson (1970a) found that germinal derivatives of the autonomous a1-m(papu) allele were also associated with transposition of En away from the locus 75% of the time. En transposes to either a proximal or distal position and approximately 25% of the transposed En's were located 6-20 map units from the A1 locus.

When a controlling element transposes into an appropriate allele, induction of mutability at the new site can be seen and mutability induced by controlling element insertion has been found at most loci where it can be detected. Ac controlled mutability has arisen at the bz1, sh1, wx, a1, and a2 loci (McClintock, 1951).

The first recognized case of En-controlled mutability was that of the pg-m allele. Mutability at the a1 locus (Peterson, 1961) and later at the wm-13 locus (Peterson, 1966a) arose in the pg-m lines. In both cases the new mutable alleles were controlled by En and in both cases the En was located at or near the controlled locus. En later transposed away from the a1 locus but the I element remained and continued to respond to the independently located En.

Controlling elements can insert into many sites along the chromosome. Estimates of the rate of transposition of En into two target loci indicate

that there may be between 100,000 and 200,000 possible insertion sites for En within the maize genome. Using an isolation plot in which A2A2CCRRA1A1 or A2A2CCRRA1a1 stocks containing En were crossed as females by a2 or c1 homozygous recessive tester males, newly arisen a2-m and c-m mutants were isolated at rates between 1.5 and 9.4 x 10^{-6} (Peterson, 1978). Based on these results of En insertion into the A2 and C genes, it would seem that there are few, if any, limits to the site of En insertion.

What are some of the limitations of controlling element transposition? Are there site, regional, or distance limitations? Some studies show that transposition occurs to sites closer to the position of origin. Twenty-five percent of the transpositions of En from the a1-m(papu) locus occurred to linked sites on the same chromosome (Peterson, 1970a). With Mp this preference for a nearby position is more apparent. In 65% of Mp transpositions from P-vv, Mp transposed to a linked region (Brink, 1958).

Controlling elements can return to their original linkage position. The rate of return of Mp to the P locus was in negative correlation to the map distance of the transposed element (Orton, 1966). Although the mutable allele can be reconstituted after transposition,the controlling element may not be reinserted at exactly the same site (Brink and Williams, 1973).

Mechanism of Transposition

There is a direct relation between the mutational activity and the transpositional activity of the controlling elements and both processes seem to be controlled by the same gene product.

This relationship can be seen in the dosage effects present in some of the controlling element systems. Dosage effects are observed in the Mp system. When more than one Mp is present the mutational activity at the receptive allele is decreased. Fewer and smaller sectors of dominant gene expressions are seen. The increased dosage of Mp also decreases transpositional activity. Early transpositions of Mp away from an autonomously controlled mutable allele will often result in ear sectors of stable germinal derivatives. When an extra Mp element is present fewer and smaller sectors of germinal derivatives are found (Wood and Brink, 1956).

A direct relationship between the mutational strength and transpositional strength of individual controlling elements is also seen. Both processes are controlled by the regulatory element and the receptor element cannot cause sectoring or transpose by itself (McClintock, 1952). Mp elements that cause earlier gene activations resulting in larger dominant sectors also give rise to earlier transpositions resulting in larger sectors of germinal derivatives (Anderson and Brink, 1952).

A similar relationship between mutation and transposition is seen in the Spm(En) system. Spm-w has a normal suppressor strength but a weak mutator. Responsive kernels with Spm-w are colorless with a few late occurring colored sectors. Spm-w undergoes few transpositions and germinal derivatives seldom arise when Spm-w is present alone (McClintock, 1957). Spm-s has normal mutator strength and can cause Spm-w transpositions. Plants with Spm-w located at the alm-1 allele were crossed by plants with Spm-s linked to Pr on chromosome 5. In the progeny,germinal mutations of

a2m-1 without Spm-w were found. Spm-w was sometimes traced to new linkage positions after exposure to Spm-s, but it continued to give its characteristic pattern of few, small colored sectors at the new locations (McClintock, 1967a).

There are several studies (Williams, 1972; Fradkin and Brink, 1956) that illustrate the independence of the mutation and transposition processes from recombination.

It can occur in all systems premeiotically causing plant and ear sectoring with no exchange of flanking markers. Transposition of Mp occurs in hypoploid stocks derived from B-translocations where crossing-over occurs at a negligible rate if at all (Williams, 1972). Hemizygous plants were derived by pollinating ears carrying the mR-nj gene under Mp control with inbred lines homozygous for translocation B-10a carrying R-g . Non-disjunction of the B chromosome resulted in mR-nj/embryos. Germinal derivatives caused by transposition of Mp away from the R-nj allele appeared in the hemizygous lines at an increased rate in the absence of any recombination.

Although Abn 10 increases cross-over rates and also the number of germinal derivatives from mR-nj, the effect must be somatic since earlier transpositions resulting in larger ear sectors of stable R-nj kernels are found (Williams and Brink, 1972). While controlling element transposition is independent of maize recombination, it may have a relation to the structure or function of the host DNA. Brink's extensive studies of Mp transposition away from the P locus have led to several models of controlling element transposition.

The P-rr allele with an inserted Mp element (P-rr Mp) will determine a medium variegated kernel phenotype. Lines of P-rr genotype with a transposed Mp have red pericarp kernels. The presence of the transposed Mp can be detected by its effect on a standard Ds tester. Light variegated kernels are found in P-rr Mp kernels with a transposed Mp (Brink and Nilan, 1952) and P-rr Mp with two transposed Mp's determines a very light variegated kernel type (Brink, 1954). All sectors of red kernels were found to be paired or potentially paired with a light variegated twin sector of equal size (Greenblatt and Brink, 1962). A transposed Mp element was found at the same linkage position in both the red and the light variegated kernels from the twin red sectors in most cases (Greenblatt, 1966). If transposition occurred after DNA replication in the parent cell it would be unlikely that the Mp element in both sectors would insert at the same position in the daughter lines. If transposition had occurred before replication there would have been no light variegated sector (Greenblatt and Brink, 1963). These authors speculated that transposition took place after the P-rr Mp was replicated but before replication of the receptor site to account for the appearance of twin sectors. This is supported by the fact that the transposed Mp occupies the same linkage sites in chromosomes of both sectors (Greenblatt, 1968). One model of Mp transposition is illustrated in Figure 1. This model (Greenblatt, 1974) based on conservative DNA replication states that:

1. Mp moves to unreplicated sites.
2. Mp replicates with the maize chromosome.
3. The newly replicated Mp, not the Mp in the template strand, transposes.

Figure 1. Diagramatic representation of all hypothetical sequences by which transposition of modulator could occur during mitoses.

A. Disposition of Mp during replicatin

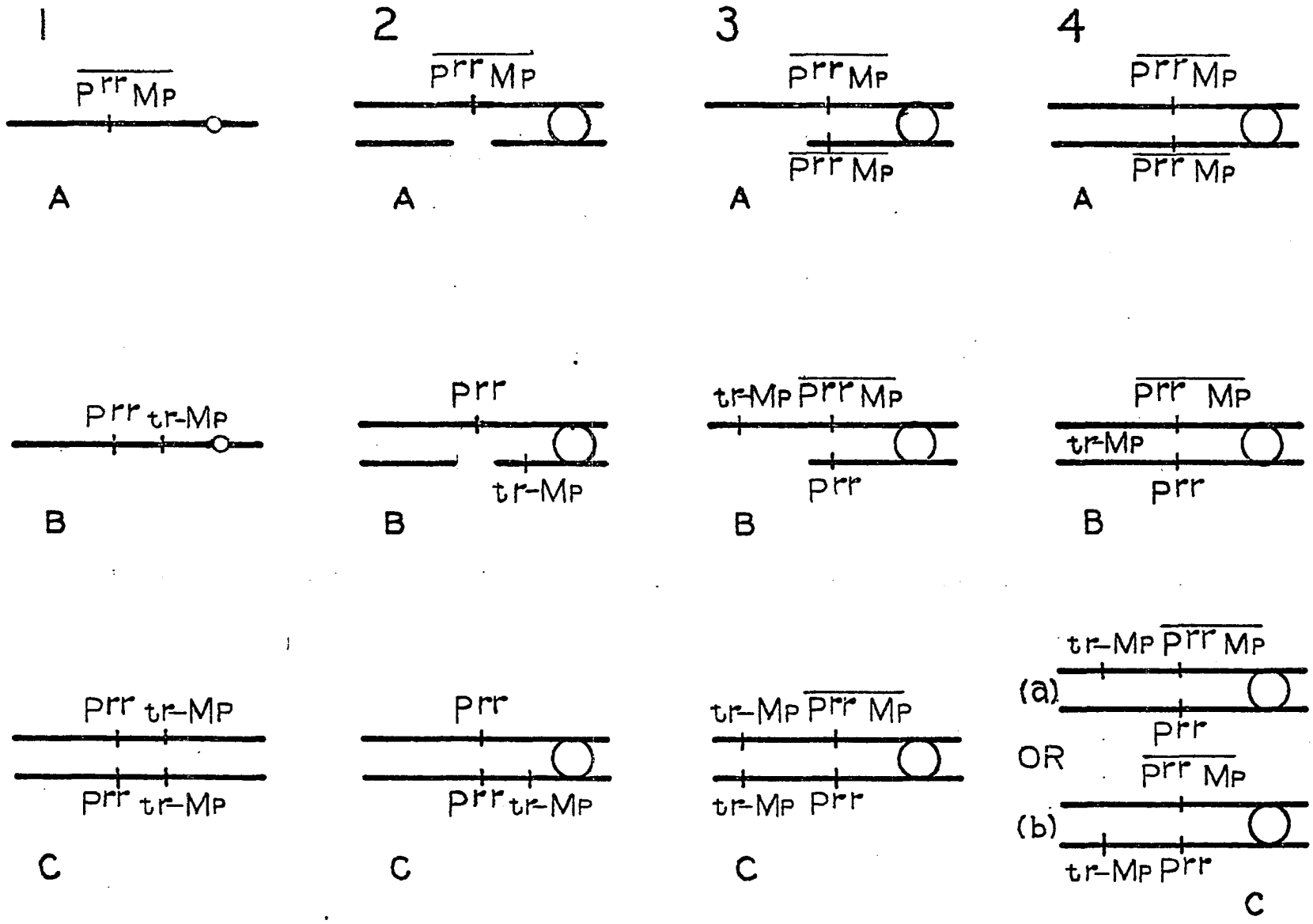
B. Location of transposed Mp

C. Completion of replication

1. result: red sector with transposed Mp
2. result: red sectors with transposed Mp
3. result: twin sectors only all contain tr-Mp
4. results:

a) twin sectors only, on transposed Mp in red sector
b) red sector only, all contain transposed Mp

(Greenblatt, 1974).



TRANSPOSITION OF MODULATOR IN MAIZE

4. Mp moves preferentially to the template strand containing P-rr Mp.

5. Sites are receptive to Mp insertion just prior to replication.

This model has been criticized by Fincham and Sastry (1974) because it is based on conservative DNA replication. Brink and Williams (1973) proposed another model based on semi-conservative replication (Fig. 2). In this model replication triggers micro-nondisjunction of the Mp element. The single-stranded Mp element displaced from the chromosome moves preferentially to a non-replicated region on the same chromosome. The transposed Mp is copied in the sister strand by a DNA repair mechanism before replication in most cases.

Two assumptions are made in both these models: 1) that DNA replication triggers transposition and 2) that the Mp element moves to an unreplicated area of the chromosome. Both models presume that twin sectors are an obligate by-product of transposition. This is not always the case, however. There are several explanations for a failure of twin sectoring. First, Mp insertion is only about 90% efficient and in the 10% of the cells in which Mp is lost, the daughter lines would develop into a red and a medium variegated ear sector. Secondly, in some ears the twinned sectors could be separated by accidents of development and in other cases the sectors may be in positions so that one of the daughter cell lines does not develop into an ear segment.

A model modified after the Greenblatt model has been proposed by Fincham and Sastry (1974). This model (Fig. 3) attempts to explain the same data on Mp transposition but does not assume twinning of sectors or

Figure 2. A chromosomal model illustrating transposition of Modulator, represented by a zigzag segment from one site to another.

A. Micronondisjunction of Mp during strand separation at DNA replication

B. Initial incorporation in single strand at new site

C. Tensional breaks occur in other strand at the same level

D. Repair

(Brink and Williams, 1973).

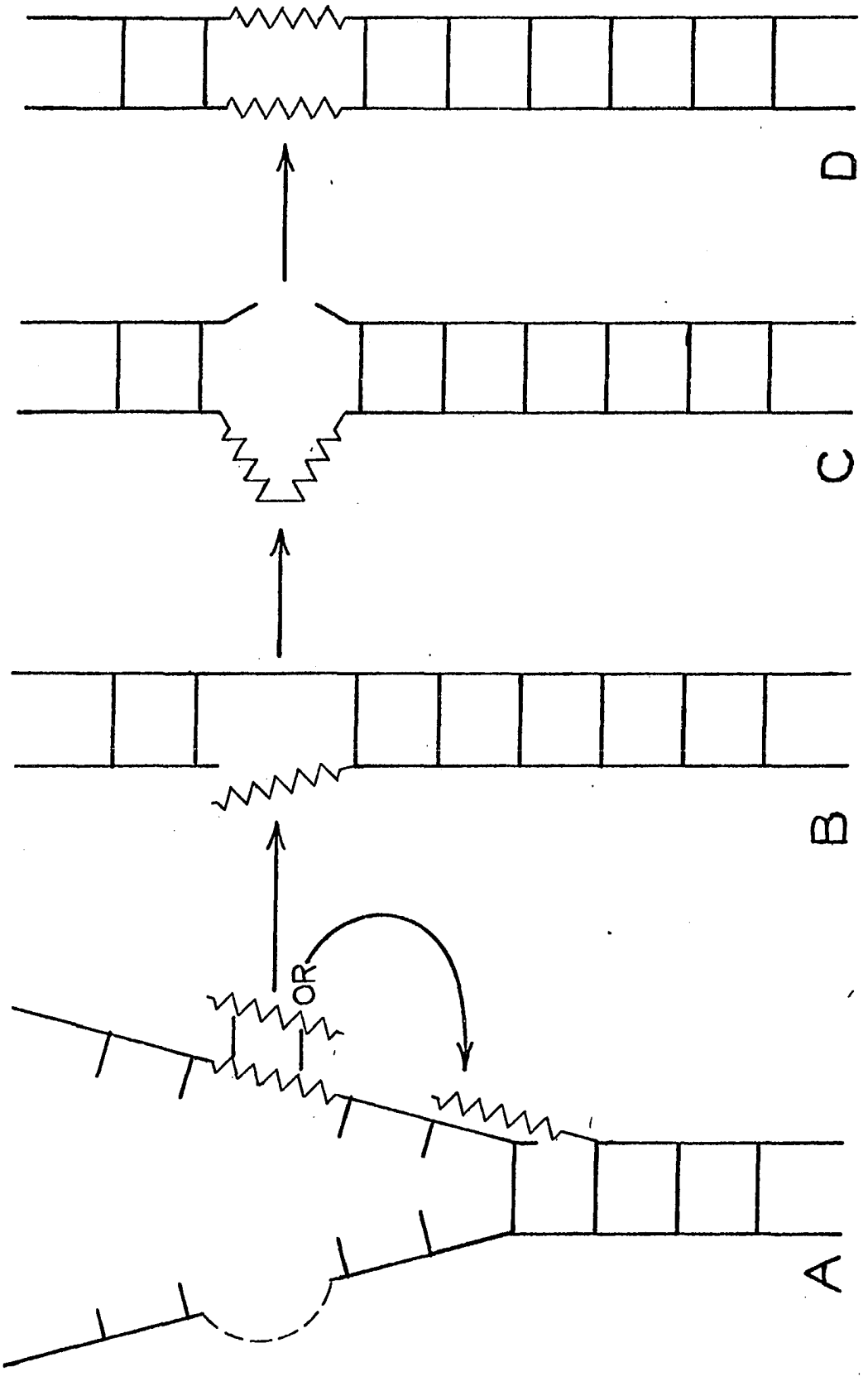

D
C
B
A
OR

Figure 3. Interpretation of Greenblatt's data on twinned and untwinned sectors on P-vv/P-ww ears.

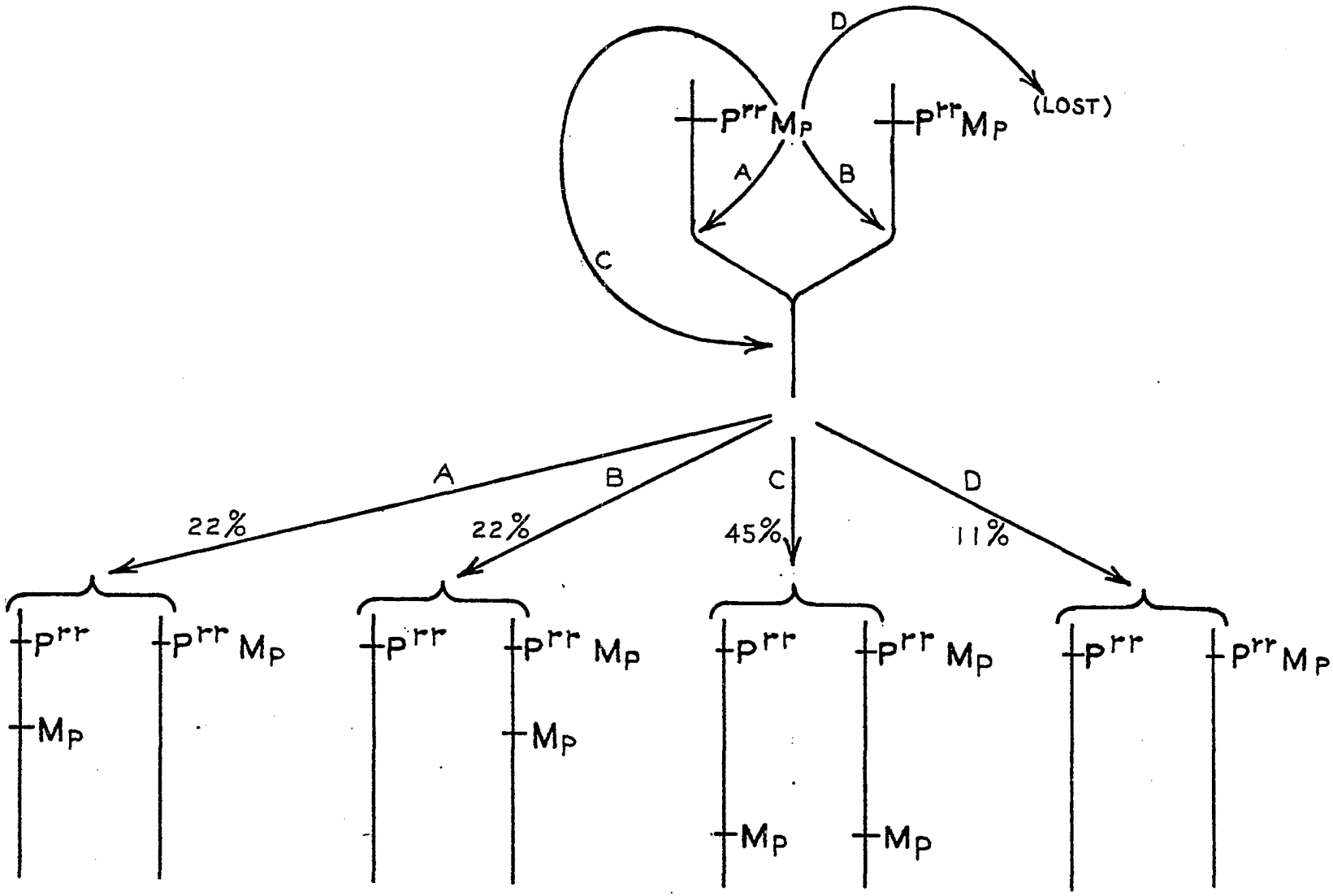
A. Mp transposes into a replicated DNA segment on same strand resulting in a red and a medium variegated sector

B. Mp transposes into a replicated DNA segment on opposite strand, resulting in twinned red and light variegated sector

C. Mp transposes into unreplicated region resulting in twinned red and light variegated sectors

D. Mp fails to reinsert resulting in red and medium variegated untwinned sectors.

(Fincham and Sastry, 1974).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M_P
P^{rr}
$P^{rr}M_P$

conservative DNA replication. In the Fincham and Sastry model Mp insertion shows no preference for replicated or unreplicated areas of the chromosome.

Changes in State

The pattern of variegation produced by mutable alleles is determined by the timing and frequency of the mutation event (Peterson, 1965b). The patterning is under precise control and is heritable, but can be affected by some factors external to the controlling element itself. Environmental factors that shorten or lengthen the duration of cell division can affect the size of colored sectors to some extent (Van Schaik, 1955).

In the Ac-Ds and al-dt-Dt systems increased dosage of the regulatory element will delay the release of gene activity resulting in smaller colored sectors (McClintock, 1953; Rhoades, 1938). En, however, shows no dosage effects (McClintock, 1965; Peterson, 1960).

Modifiers of controlling element activity have been identified in all of the systems under study. The modifier and restrainer genes have no apparent effect except for their enhancement or restraint of the controlling element. They may represent a secondary level of integration (McClintock, 1957; Peterson, 1976a).

The regulatory element and also the receptor element in the presence of the regulatory element can undergo changes in state resulting in new patterns of kernel variegation. The pattern produced will depend on the state of the receptor element, the state of the regulatory element, and their interaction. Changes in state are heritable and independent of modifiers and dosage effects (McClintock, 1967a).

States of the I-En system can range in pattern from single-celled colored sectors in the a2-m-1629 allele (Peterson, 1976b) to nearly completely colored kernels in the a1-m (dense) allele (Peterson, 1979). McClintock (1955a) has catalogued a series of receptor states at the A1 and A2 loci under Spm control.

These states differ in their phenotypic expression among each other both in the presence and in the absence of Spm. In the absence of Spm the states range from completely colorless to various grades of solid coloration from pale to fully colored. They also differ in their level of activity in different plant tissues and in their response to both the suppressor and the mutator functions of Spm. In the heterozygous condition each receptor element will respond to Spm in its own characteristic pattern of mutability and the two patterns are superimposed (McClintock, 1955).

Some specific examples will illustrate the wide diversity of gene expression inherent in the states of receptor elements at a single locus. One state of the a1-m-2 allele is colorless in the absence of Spm but responds to the suppressor in Spm by producing a uniform pale coloration (McClintock, 1967b). Response to the mutator function is normal. The resulting pattern in the presence of Spm is a pale aleurone with spots of darker coloration. In contrast, the a1-m-1 state, the aleurone is pale in the absence of Spm and colorless with dark spots in the presence of Spm (McClintock, 1965).

State of controlling elements can respond to the internal environment of the plant. In the a1-m(crown) allele, the base of the kernel is colorless while the crown is spotted. With a1-m(flow) the opposite

expression is seen and the base only is spotted (Peterson, 1966b). In the heterozygote the states respond independently, each producing its own characteristic pattern of mutability, and spots are shown at both the crown and the base of the kernel.

Changes in state can also occur in response to the tiller environment. In some lines, the pattern of mutability in the tiller ear is different from the pattern produced by ears of the main stalk. The change in state induced in the tiller ears is permanent and heritable (Fowler and Peterson, 1978).

Both Ac and Spm undergo cycles of activation and deactivation (McClintock, 1958). The effects of cyclic deactivation of Spm can be easily observed at the a2-m-1 allele.

Two states occur at the a2-m-1 allele. The first state behaves like most responsive alleles. It is pale in the absence of Spm, but in its presence, produces kernels with spots of color on a colorless background. The spots of color are produced by the mutator function of Spm. The behavior of the second state is unique. In this state, a2m-1 is fully colored in the absence of Spm but in its presence, the spots on the kernels are pale colored on a colorless background. Each pale-colored sector is the result of the deactivation of the Spm element. This deactivation is confirmed when a2-m-1/a2-m-1 wx-m-8/wx-m-8 kernels with Spm are examined. The pale-colored spots in this genotypic arrangement overlay regions of endosperm with no activity of the Wx gene. If the pale-colored sectors were due to release of gene activity mediated by the mutator product a corresponding release of the wx-m-8 gene would have been

seen. In some cases, Spm will be reactivated and then again suppressed producing sectors of colorless tissue with spots of color within a larger colored region of the kernel. These cycles of activation and deactivation can last through successive sexual cycles.

Pale kernels from a2-m-1 (state I) can produce plants with completely pale ears containing an inactive Spm, pale ears with a few mutable kernels, or ears with normal segregation of mutable kernels reflecting a fully active Spm. In some cases, the three types of ears can be seen on the same plant.

The presence of an inactive Spm in the pale kernels can be detected by its dosage effect in combination with an active Spm. While Spm normally shows no dosage effect the presence of more than one Spm in a2-m-1 will result in smaller and fewer colored sectors since all of the Spm's present must be deactivated for color to be produced. When an active and an inactive Spm are present together the pattern is the same as that produced by two active Spm's (McClintock, 1970). The active Spm can reactivate the inactive Spm when both are present in the same cell but when the two are separated by segregation at meiosis the inactive Spm will again become quiescent. Pale kernels containing the inactive Spm will appear in the progeny. Spm must normally code for an activator substance missing in the inactive Spm.

While a2-m-1 (state II) does not respond to the mutator product of Spm by gene activation, it will undergo changes in state only when an active Spm is present.

Cycle changes in activity can also be observed in the a1-m(Au) allele (Peterson, 1979). However, in this allele the suppressor and mutator undergo independent cycles of activation and deactivation.

Models of Controlling Element States

The state of a controlling element seems to depend on its position within the genome and in many cases changes in state are associated with transposition.

When En is located at the a2-m4 1629 allele, mutability is expressed as one cell sized colored spots on a colorless background and the autonomously located En has a very weak effect on a1-mr or a1-m-1 tester lines. Following transposition the En gives a normal tester response (Peterson, 1976b). The a1-m(flow) allele is also autonomous and spotting is restricted to the base of the kernel. When a1-m(flow) lines are crossed by lines carrying other states of En, but no responsive allele, evenly distributed spots are produced in the progeny kernels (Peterson, 1966b). After En transposes away from a1-m(flow) it will produce evenly distributed spots on a standard responsive tester.

In a study of 18 a2-m and 30 c-m newly arisen autonomous alleles, it was found that the patterns of the newly arisen mutable alleles showed no correlation to the pattern produced by the original En on a standard tester allele (Peterson, 1976b).

Similar results were found in studies of reconstituted mR-nj alleles. Transpositions of Mp away from and back to the R-nj allele were selected. No correlation was found between the original Mp produced on a standard

tester line and the pattern produced at the reconstituted mR-nj allele (Brink and Williams, 1973).

These results led to the position hypothesis for the state of controlling element mutability (Peterson, 1976a). The state of a controlling element depends on the position of insertion within the genome and is not an inherent quality of the controlling element itself.

Controlling Elements in Other Species

Systems of inherited instability are common in plant species particularly in the ornamentals. In cases where the genetic control of the instability has been studied it is often found to be similar to controlling element systems in maize.

Variegation in Antirhinum flower color is due to a high mutability at the pallida-recurrens (pal-rec) allele (Fincham and Harrison, 1967). As in maize, changes in state often occur and stable derivatives are generated. Although mutability has arisen at other loci in pal-rec lines, there is no definite evidence that a factor at pal-rec has transposed into the new mutable allele (Harrison and Carpenter, 1973).

Controlling element-like systems have appeared in interspecific crosses among Nicotiana tabacum, N. sanderae, N. otophora, and N. langsdorfii (Sand, 1969; Gerstel and Burns, 1967; Smith and Sand, 1952). The controlling element originating in the N. langsdorfii x N. sanderae hybrid was autonomous and located at the V locus determining flower color. A modifier of v-m arose in the mutable lines and was traced to a separate site linked to the E locus (Sand, 1976). Like controlling element systems in maize, new mutables have appeared at other

loci, stable derivatives arise from the unstable alleles, and changes in state occur (Sand, 1976).

Mutable alleles that have many parallels to maize controlling elements have also been found in Drosophila (Green, 1969), in yeast (Egel, 1977), and in bacteria. Controlling elements are not restricted to maize but are a widespread phenomena.

Analogies to Bacterial Systems

Controlling elements in maize have many features in common with the transposable elements in bacteria and these transposons and insertion sequences provide the best molecular models for controlling element action in eukaryotes (McClintock, 1961; Peterson, 1970b; Nevers and Saedler, 1977). Like controlling elements in maize, transposable elements can insert into genes blocking activity. Excision or inversion can restore the gene to its normal functional condition. They can be mapped at many locations in the bacterial genome. They can cause deletions resulting in a lower or a null gene expression similar to stable germinal derivatives of controlling element systems. The insertion sequence (IS) elements are small inverted or directly repeated sequences 800-1400 base pairs in length (Starlinger and Saedler, 1976). They may contain genes for their own excision and insertion and must also contain some promotor genes but they code for no other known functions.

The IS elements contain a nonsense codon followed by a rho terminator sequence and when inserted into a bacterial gene will block transcription. The result is a polar mutant of the host gene (Starlinger and Saedler, 1976). The IS elements can insert in two orientations. In IS2 a promotor

allows gene expression in orientation II while gene expression is blocked in orientation I. The IS2 element can act as a gene switch by a "flip-flop" mechanism. New promotors have been found in IS2 in orientation I that give intermediate constitutive levels of gene activity similar to the a1-m-1 allele in maize (Saedler, 1977; Peterson, et al., 1979).

Deletions are generated in material adjacent to insertion elements, IS1 and IS2, at 100-1000 x the rate of spontaneously generated deletions (Nevers and Saedler, 1977; Peterson, et al., 1979). The end points of the deletions terminate at the IS site on one end but can be located at various, but non-random, points at the other.

Transposons are often bordered at each end by identical sequences of known IS elements in either direct or inverted order. Transposons can insert into areas in the bacterial chromosome and also into plasmid DNA. Bacterial genes can become integrated into a transposon and can be introduced into new sites within the host DNA (Shapiro et al., 1977).

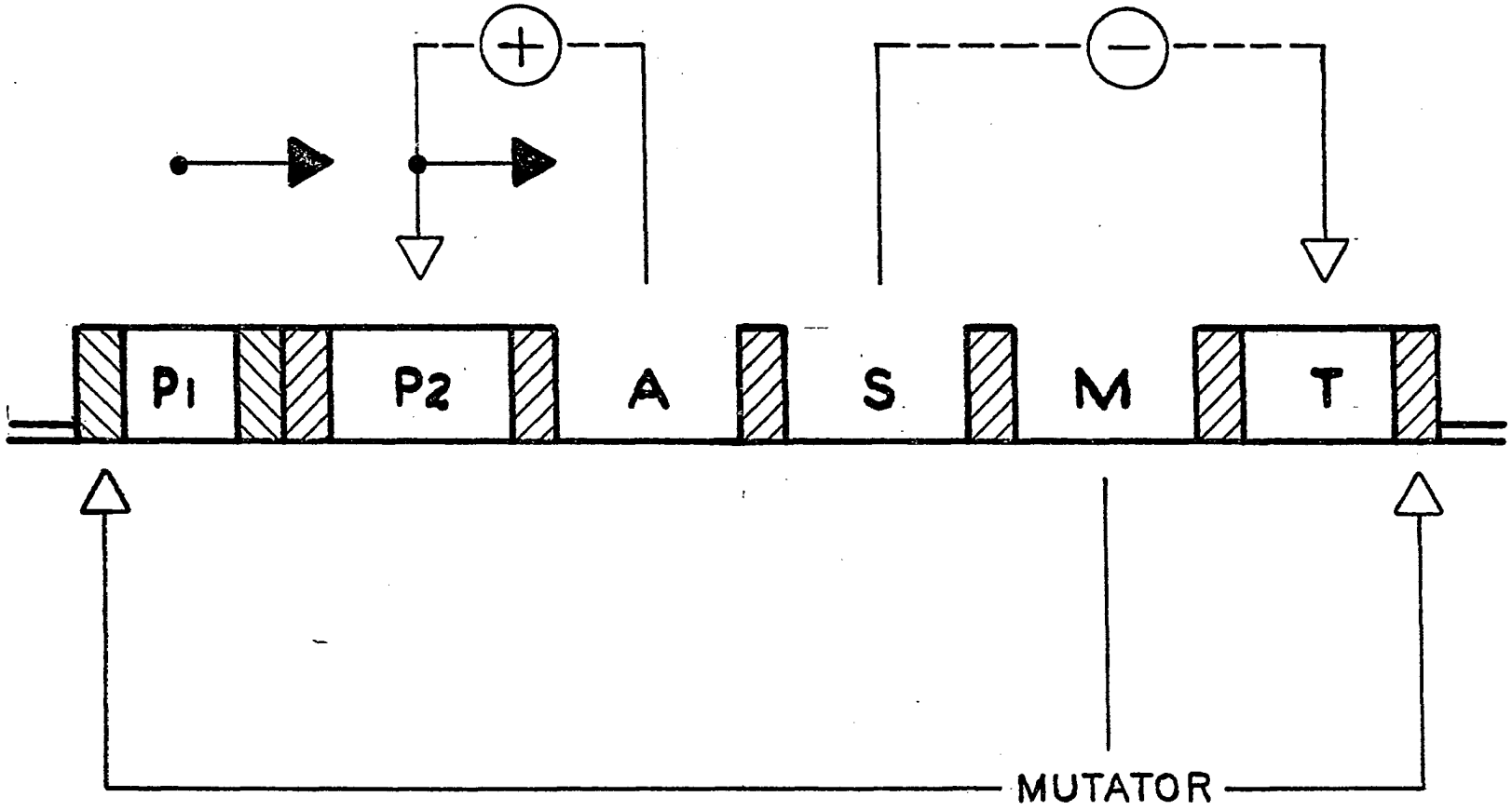
Insertion of IS elements, transposons and temperate phages occurs by a process of "illegitimate" recombination and will occur in rec A- bacteria, defective in the ability to carry out homologous recombinations. In some transposable elements sequence homology between the inserting segment and the host DNA is required while in others no apparent homology is needed. In the case of λ phage of E. coli, insertion and excision depend on two phage genes int and xis and regulatory sites for these genes are known (Landy and Ross, 1977). During integration the phage circularizes. Integration occurs at corresponding sites called POP' in the phage and BOB' in the bacteria. The O region is common to

both phage and bacteria. It is found to be a palindrome, 15 BP's long. The O region must be a repetitive sequence in the bacteria because based on the number of integrative sites the O region was expected to be about 6 base pairs in length. The bacteriophage Mu seems to integrate completely at randpm (Bukhari, 1976). While the transposon Tn1 restricts itself to some regions of its host DNA, it seems to require no specific base pair sequence for insertion within these regions (Weinstock, et al., 1979).

A model of Spm(En) structure and function (Fig. 4) has been proposed based on the data accumulated on bacterial insertion elements (Nevers and Saedler, 1977). According to this model, the suppressor and mutator functions act independently in Spm and their functions are assumed to be coded in separate genes. The model also contains a promotor and a terminator site. The sites of gene activity of the Spm model are separated by small black box sequences thought to be similar to the IS elements. These are the sites of excision and integration. According to the Nevers-Saedler model, the I element consists of a terminator sequence bordered by two black box sequences. This segment can be left behind when an originally autonomous Spm transposes. It can respond to the suppressor and mutator gene products. The promotors of Spm can respond to signals produced by the maize plant by activating at a precise stage of development as in the al-m(flow) and the al-m(crown) alleles.

Both P1 and P2 can be present in reverse or direct orientation and their inversion is proposed to control the cyclic activation and deactivation of the Spm element (McClintock, 1958). Both P1 and P2 must be in

Figure 4. Proposed structure and function of active Spm. A model of the molecular structure of an integrated Spm regulator element is illustrated. P1 and P2 are promotor units with the direction of transcription indicated by arrows above them. T is the terminator sequence. The A (Activator), S (Suppressor), and M (Mutator) are encoded in structural genes. The action of these products is depicted by dashed lines. Recombinational events occur at the cross-hatched "black box" sequences.



STRUCTURE AND FUNCTION OF ACTIVE SPM

direct orientation for Spm activity. If P1 is inverted but P2 is direct, Spm is turned off and the Spm is inactive. However, the P2 promotor can be activated by the A gene product from an active Spm. In this way, an active Spm can cause trans-activation of an inactive Spm as observed by McClintock (1970) at the a2-m-1 allele. The entire S and M genes may be able to invert also resulting in independent cycles of activation and deactivation for these two functions (Peterson, 1979).

Developmental Role

The occurrence of controlling elements or their counterparts in such widely divergent species as maize, Drosophila, and bacteria raises speculation about their biological significance. Although maize controlling elements have no deleterious effects they have no known developmental function. It may be that their presence only becomes apparent through their abnormal effects when out of their usual role in the plant. They have often appeared after some disturbance in the genetic functioning of the cell such as bridge breakage fusion or irradiation (McClintock, 1947; Peterson, 1953; Doerschug, 1973).

Similar genetic factors in other species are known to provide a selective advantage for the affected genotype. A controlling element in yeast is responsible for determining mating type (Egel, 1977). Mutability for this trait insures that both mating types will be present in isolated colonies. Antibody variability may also be due to a controlling element like factor (Shapiro et al., 1977).

In Salmonella an inverting chromosomal segment controls flagellar type. The bacteria can switch from one flagellar type to another as this segment inverts (Zieg, et al., 1977). In this way, chromosomal rearrangements can provide a means of genetic control.

Controlling elements in maize can have two possible functions. They can act as a source of variability and also function in gene regulation and cell differentiation. By causing rearrangements of the chromosome, controlling elements will bring new combinations of nuclear sequences into proximity and will sometimes join structural genes to new promotor regions providing previously untried pathways of genetic control. Rearrangements of functioning nuclear sequences is a more efficient method of generating variability than point mutation.

Controlling element insertion and excision can also provide a wide range of control of genetic expression as evidenced by the many controlling element generated aleurone patterns.

It has been suggested that controlling elements are vestiges of episomes that became incorporated into the genetic mechanism of their host in the same way that mitochondria may be vestiges of bacterial infections that have become integral parts of the host cell (Darlington, 1978). It has been observed that controlling elements can direct the time during development when genes act and the tissues where specific genes will be active. They can also determine the level of gene activity. And, more than one gene may be regulated by the same element. The elegance of their regulation and their pervasiveness in the maize species argues for an, as yet unknown, but specific role in the life of the plant (McClintock, 1967b).

MATERIALS AND METHODS

Gene Symbols

al-m(r)	Recessive allele of Al, responds to En. The aleurone is colorless in the absence of En. Dark spots on a colorless background are produced when En is present (Peterson, 1961).
al-m-1	Recessive allele of Al, responds to En. In the absence of En the aleurone is pale; colorless with spots of full color in its presence.
al-m(nr)	A derivative of an originally autonomously mutating allele that has become stable through the loss of En (Peterson, 1966c).
al-m(pa-pu)	An autonomously mutating allele of the En controlling element system. The aleurone is colorless with both pale and full colored spots (Peterson, 1961).
al-pm	An autonomously mutating allele of the En controlling element system. The aleurone is pale with fully-colored spots (Peterson, 1961).
al-m(Au)	An autonomously mutating allele of the En controlling element system. The aleurone is nearly full colored but with some colorless sectors (Peterson, 1961, 1979).
et	Etched-etched markings on the aleurone surface; a marker gene 13 map units distal to al (Neuffer et al., 1968).

Source of Materials

Three autonomous mutable alleles of al were used in this study of the transposition mechanism of En. The original En was found in a pale green mutable (pg-m) plant among Bikini exposed material (Peterson, 1960). Among outcrosses of pg-m, the al-m allele was observed as a mutable tassel sector in an Al/al-dt plant (Peterson, 1961). Dt was not present in the line and linkage studies indicated that the Al gene had mutated to al-m. In outcrosses of this al-m, numerous exceptional pattern types arose distinguishable by the quality and quantity of gene mutability. Among this array, three mutable alleles al-m(Au), al-m(pa-pu), and al-pm were utilized in further studies of En changes.

These three alleles are autonomously mutating with En located at the al locus and each has a very distinctive pattern of mutability (Figs. 5, 6, 7). The al-pm allele produces pale coloration in the aleurone with dark sectors. Kernels with al-m(pa-pu) have both dark and pale sectors on a colorless background. The al-m(Au) allele is phenotypically indistinguishable from the standard Al except for large colorless areas in the aleurone layer.

These three alleles were chosen for this study because of their distinctive differences and numerous derivative changes. This will permit comparisons of transpositional activity among En's.

Detection of En Transposition From al

Colorless and pale non-responsive derivatives (al-nr) indicating a loss of En at the al locus were isolated from the three autonomous

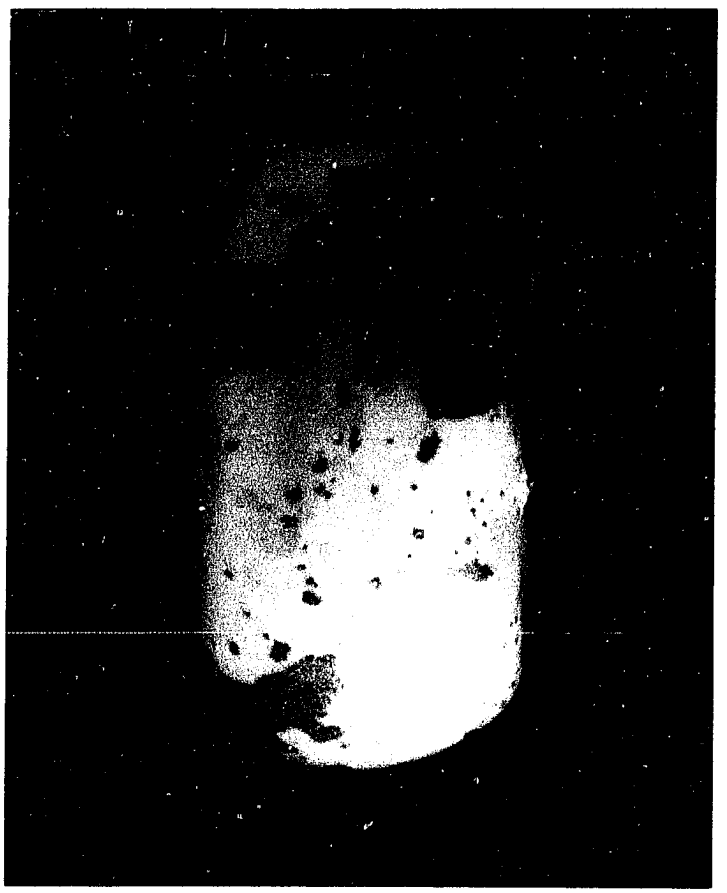


Figure 5. al-m(papu).

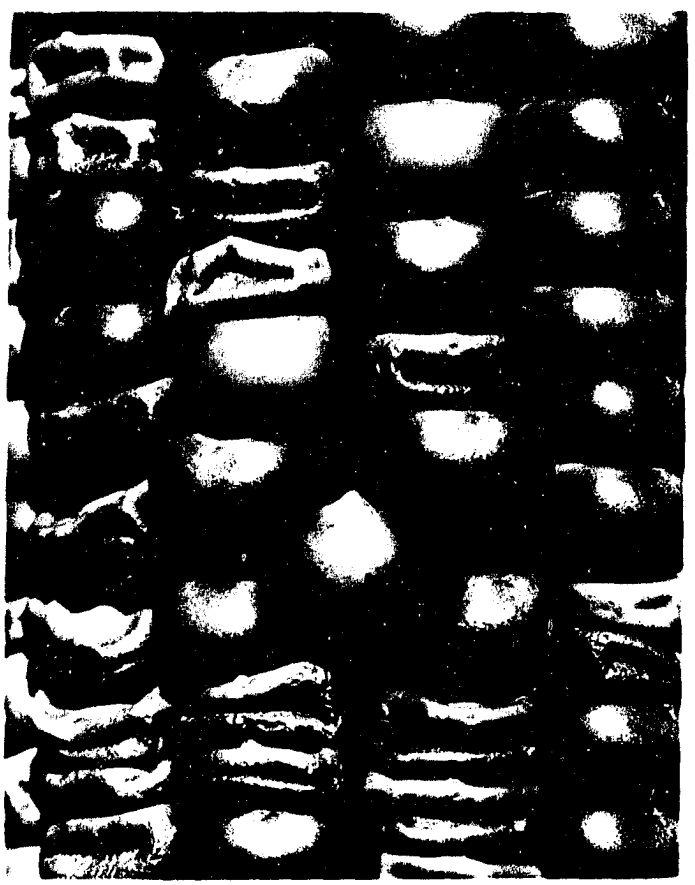


Figure 6. al-pm.

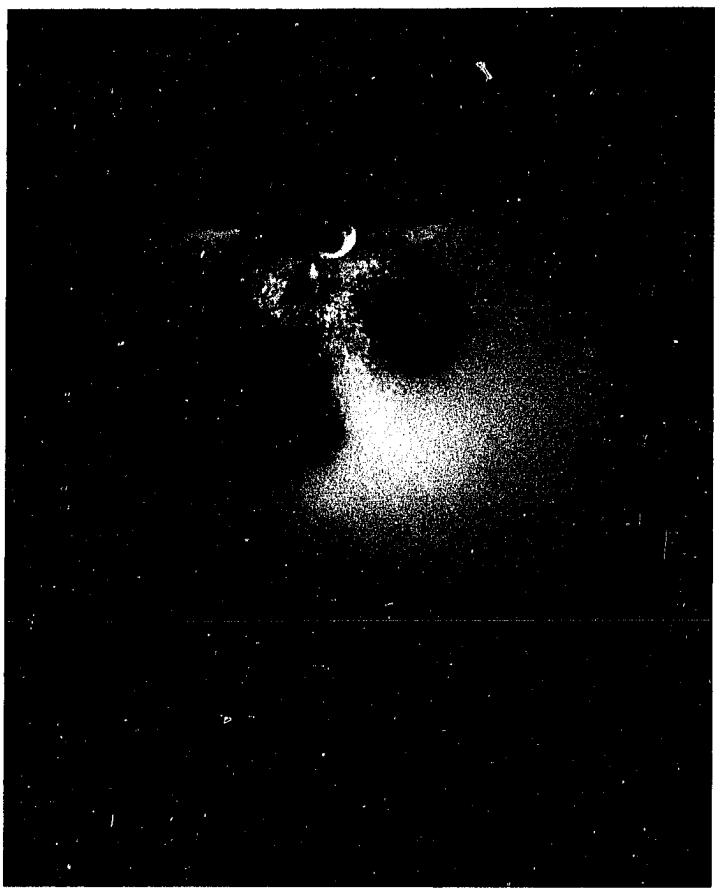


Figure 7. al-m(Au).

source lines in cross 1 (Table 1). Round, stable kernels indicate a change at the al-m locus since the sh2 allele is closely linked to al and this obviates a cross-over origin. The pale and colorless derivatives were crossed to an al-mr/al-mr tester line to determine the presence of En (cross 2). Spotted kernels from cross 2 indicating the presence of En were crossed to an al et tester to determine the linkage of En to al (cross 3). Progeny with 25% mutable kernels among the total would indicate an independent En. If fewer than 25% mutable kernels are found among the progeny of al-m(nr) in cross 3, En is linked to the non-responsive allele since all mutable kernels would result from cross-overs between al-m(nr) and En. Linkage was confirmed and the position of En relative to the centromere was determined by backcrossing a twelve seed sample from each ear in cross 3 with a linked En to the al et tester line (cross 4).

Each of the ears from cross 4 produced five classes of kernels:

1) spotted, smooth
2) spotted, etched
3) colorless, etched or smooth
4) pale, smooth
5) pale, etched

The linkage value was calculated as:

% recombination = 2(50% - % spotted kernels)

Calculations were made on this locus since:

50% - % spotted = 1/2 of recombinants

1/2 of % recombinants = total % recombination

Table 1. Determination of linkage position of transposed En

cross 1 $\frac{\text{al-m(pa-pu) Sh}}{\text{a sh}} \times \frac{\text{a sh}}{\text{a sh}}$

1/2 sectored round kernels, 1/2 colorless shrunken kernels exceptional round pale or colorless types.

cross 2 $\frac{\text{al-m(nr) Sh En}}{-} \times \frac{\text{al-m(r) Sh}}{\text{al-m-1 sh}}$

spotted round kernels will be: $\frac{\text{al-m(nr)} \quad + \quad \text{En}}{\text{al-m-1 or al-m(r)} \quad -}$

cross 3 $\frac{\text{al-m(nr) Et En}}{\text{al-m-1 Et} \quad -} \times \frac{\text{al et}}{\text{al et}}$

all spotted round kernels from cross-overs

cross 4 $\frac{\text{al-m-1 Et En?}}{\text{al et}} \times \frac{\text{al et}}{\text{al et}}$

Linkage was calculated for each ear in the line and on a line average basis. For those states of En with few spots per kernel linkage was calculated as:

$$\% \text{ recombination} = \left(\frac{2X \text{ no. of colored kernels}}{\text{total no. kernels}}\right)$$

Pale colored kernels result from separation of the al-m-1 allele from En and represent one-half of the cross-over events. Linkage was calculated by these formulas because not all kernel classes could be accurately distinguished.

The distal or proximal position of En relative to al was determined from the proportion of spotted, etched kernels in the progeny lines (Table 2). These kernels result from double cross-overs and the number will depend on the distance of En from al. For example, if En is 20 map units proximal to En the expected number of spotted, etched kernels will be:

$$\frac{\text{En} \quad \text{Et} \qquad \text{al-m-1}}{\text{et} \qquad \text{al}}$$

P = P (C.O. Et to al-m-1) x P (C.O. Et to En) P = Probability

= (.12) (.20) = .052 or 5.2%

Chromosomal Region

The al locus is positioned on the long arm of chromosome 3,102 map units from the centromere. The etched gene is 13 map units distal at 115. En could be traced to any position linked to al, in an area that includes, approximately, the distal two-thirds of 3L. The most distal marker gene

Table 2. Determination of En position distal or proximal to al

Position of En		% spotted etched
	50	2.4
	40	1.76
	30	1.10
	25	.18
	20	.45
	15	.15
distal	13	0
	10	1.35
	5	3.8
al	0	6.5
	5	6.17
	10	5.85
proximal	15	5.52
	20	5.20
	25	4.87
	30	4.55
	40	3.90
	50	3.25

is located at 121 map units from the centromere. Rhoades and Dempsey (1966) mapped an inversion breakpoint at 123 map units with 5% of the remaining part of the chromosome distal to that point. In this study, the end point was assigned at 24 map units from al since few En's mapped farther out along the chromosome. When recombination was calculated at more than 46%, En was assumed to be independent. Transpositions of less than 4 map units could not be detected using these methods. Linkage values greater than 46% would not be significantly different from 50% in most cases although the minimum transposition that could be detected depended on the number of ears and number of kernels in each line. Some En positions may be found between 46-50 map units from al and sampling error would have resulted in linkage values less than 46% for some independent En's - the cut-off value of 46% seemed to be the best compromise.

Ears that contained more than one segregating En would give linkage values of 50% for ears descended from kernels with one En, 25% for ears from 2 En kernels, and 15% for ears from kernels with 3 En's. Lines with ears that appeared to segregate for these linkage values were assumed to have more than one En and were eliminated from the study since the line averages would give false linkage values. These lines resulted from En transposition during replication of the maize chromosome and not from independent En replication.

Detection of Secondary Transposition

Chi-square tests for uniformity of linkage values of ears within each line were calculated by computer analysis. If the χ^2 values

were significant at the 5% value, the ear with the linkage values with the greatest deviation from the line average was eliminated and χ^2 values recalculated until a non-significant value was reached. Twelve seed samples were taken from deviant ears and the seeds were tested in progeny rows. The linkage values of the deviant progenies were compared to the parental line and to the deviant ear using χ^2 tests to confirm a secondary transposition. Sample data and procedural computations are presented in Table 3.

Frequency distributions were determined for all linkage values, linkage values within each of the three autonomous sources, parental sites giving rise to transpositions, sites of newly confirmed secondary transpositions, stable sites not giving rise to transpositions, and linkage sites of primary and secondary transpositions. Comparison of equality were made using Chi-square contingency tests (Snedecor and Cochran, 1973).

Determination of Change in State

Patterns were determined visually by comparison to a set of standard kernel types. This determination, based on the timing of mutation (size of spots), was made by dividing the kernel types into five classes from 10 to 50 (Fig. 8). Frequency of mutation (number of spots) was rated from 10 to 100 (Fig. 9) (Peterson, 1976a). Frequency and timing were both correlated to linkage using a general linear model analysis of variance (Snedecor and Cochran, 1973).

Table 3. Sample data and procedural computations to confirm secondary transpositions

A. Parental line 6 0848

Ear no.	No. spotted/total no. kernels	C.O. value
1	34.38	31.24
3	38.20	23.60
5	31.85	36.30
7	47.18	5.64*
9	34.47	31.06
2	45.13	9.74*
4	34.92	30.16
10	31.78	36.44
6	34.55	30.90
8	30.10	39.68

parental line cross-over value = 2(50-34.08) = 31.89

B. Progeny line from 6 0848-2

Ear No.	No. spotted/total no. kernels	C.O. value
1	44.02	11.96
2	38.60	22.80
3	37.56	24.88
4	38.57	22.86
5	38.35	23.30
6	35.56	28.88
8	46.67	6.66

progeny line cross-over value = 20.46*

*Significantly different from parental line average cross-over value at 5%.

Figure 8. Variability of timing of En mutation as reflected in the size of colored sectors. The kernels above were rated on a scale from 10 to 50 with 10 on the far left.

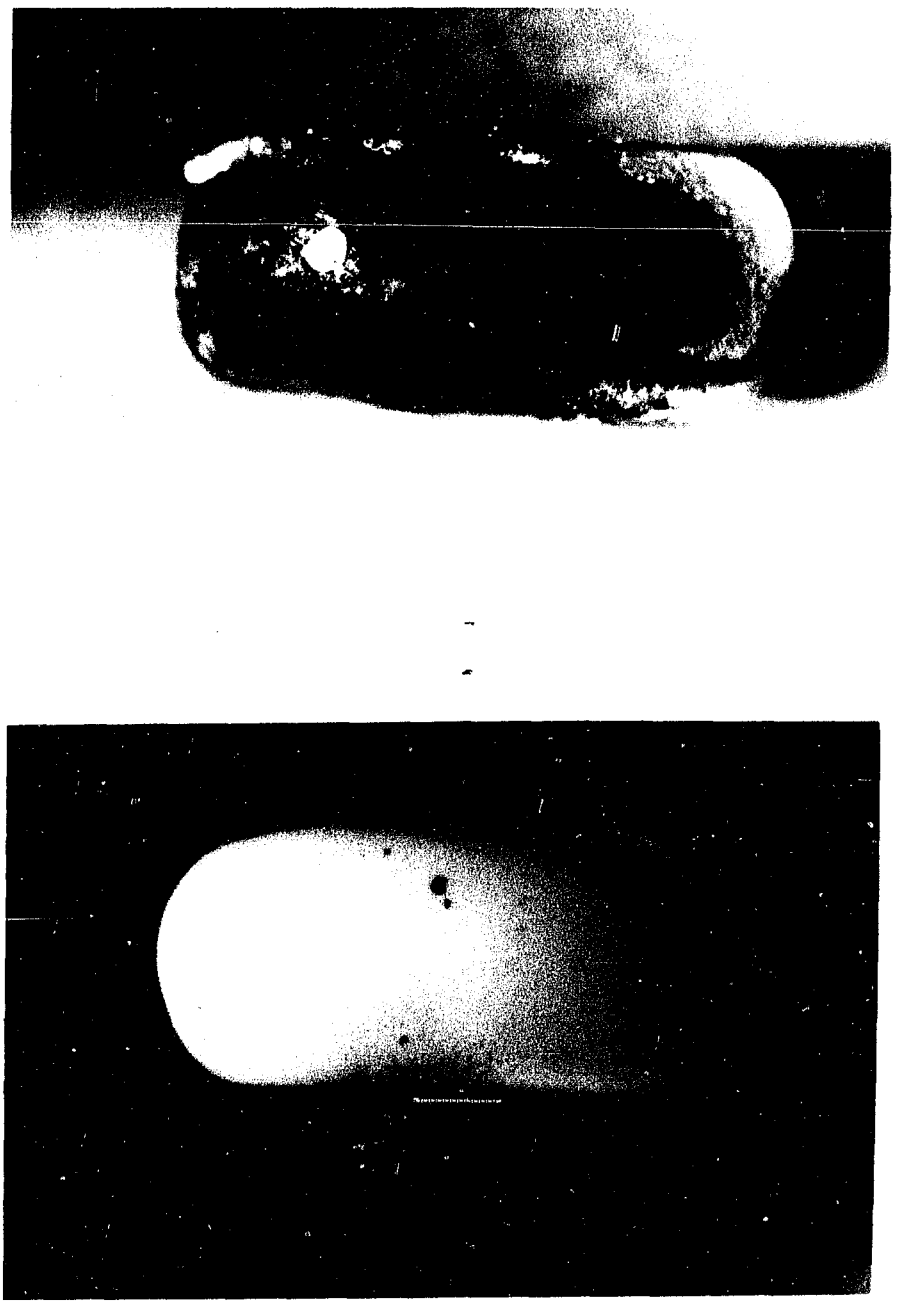


Figure 9. Variability of frequency of En mutations as reflected in the number of spots per kernel. The lower kernel was scored as 10 on a scale of 10-100. The upper kernel was scored at 100.

RESULTS

This study is focused on the process of transposition of the Enhancer (En) controlling element. Several questions on this problem have been posed. Are there preferred regions of En insertion along a portion of the 3L maize chromosome? Do all En's behave similarly in their preference or avoidance of chromosomal regions? Are En's in some regions of the chromosome more active in excision or more stable? How are En's distributed in subsequent transpositions? Is En movement limited in distance or direction along the chromosome? What is the effect of En position on the timing and frequency of mutation produced on standard mutable alleles?

In order to answer these questions,the destination of En's transposing from three autonomous mutable alleles were followed through primary and secondary transpositions.

From testcrosses of the three mutable, source alleles, stable germinal derivatives representing the expected loss of En at the al locus were selected (Table 1, cross 1). From crosses to receptive tester lines (Table 1, cross 2), transposed En's could be determined to be either linked to al or located at some independent position (Table 1, cross 3). In some lines there was evidence that more than one En was present based on the segregating linkage values of progeny rows. Linkage positions could not be determined in these lines and they were not tested further. Ear progenies with linkage values deviant from the parental line averages were selected from lines containing an En linked to al as shown in Table 3. These are identified as primary transpositions.

Kernels from these deviant progenies were tested to confirm secondary transpositions (Table 3, Part B).

About 200 primary transpositions from al were mapped and from these primary transpositions 1627 lines representing possible secondary and tertiary transpositions were tested (Table 4). The fate of the En's in the progeny line was as follows:

29%	En had undergone a confirmed secondary or tertiary transposition.
28%	No confirmed secondary or tertiary transposition.
21%	Lines showed evidence of presence of more than one En.
12%	En was confirmed to be at an independent position.
10%	Position of En could not be determined because of problems of En transmission or poor gene expression.
100%	

Distribution of Transposed En from Three Different Unstable al Alleles

Are certain regions of the chromosome preferred sites of En insertion? This is illustrated by the graph of all the En transposons linked to the al locus (Fig. 10). Linkage values of lines that were found to be significantly different in linkage value from the parental lines as well as those not significantly different are included in this graph.

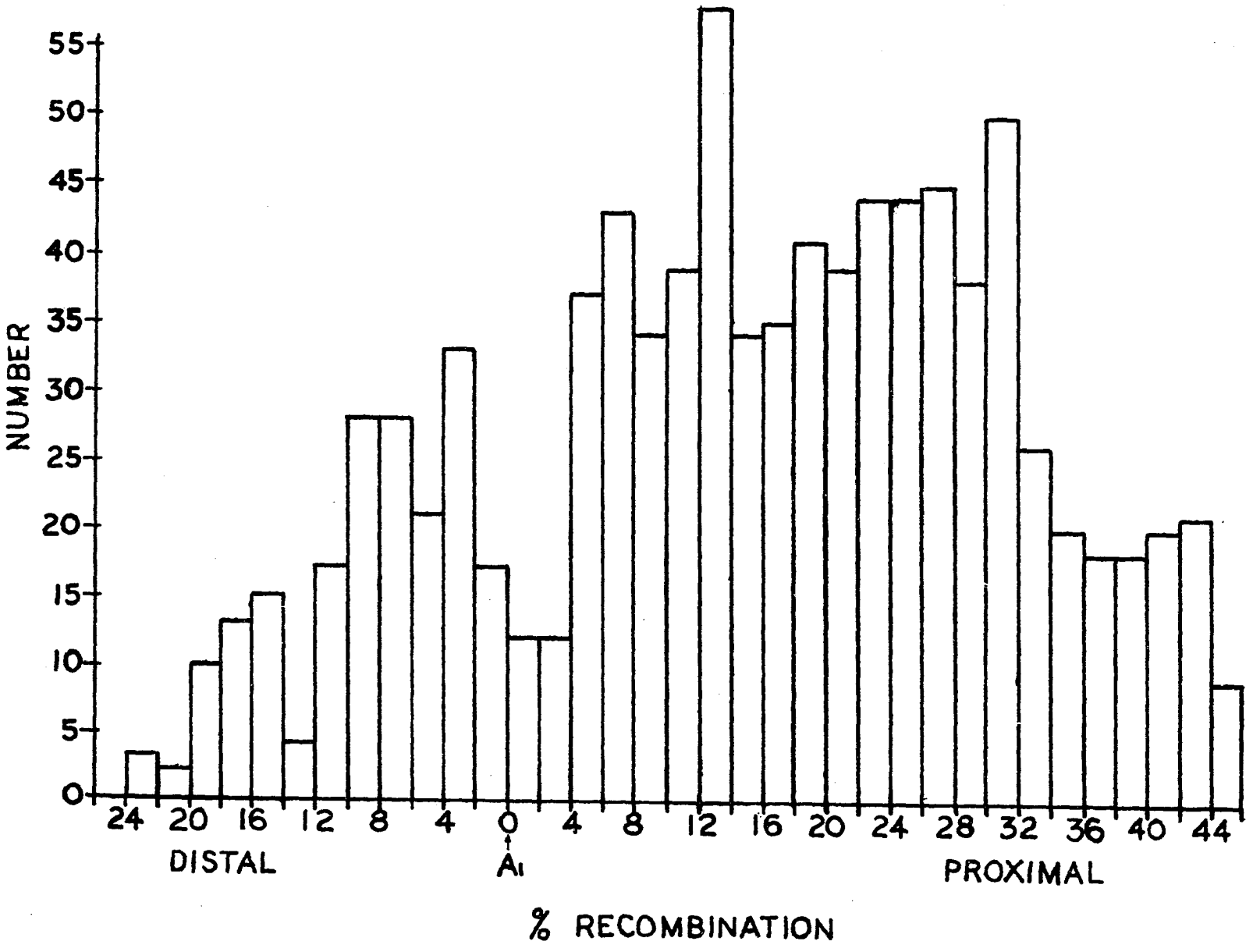
While the distribution of linkage positions was not uniform, all regions of the chromosomal segment studied had some En insertions.

Table 4. Results of progeny row tests of deviant ears isolated from *En* transpositions from three originally autonomous source alleles with *En* in both primary and secondary transposition sites

	Total	Confirmed linkages	Indep.	2 + En	Linkage change not confirmed
a1-m(pa-pu)	1212	347	166	281	324
a1-pm	332	92	23	55	101
a1-m(Au)	83	29	8	8	35

Linkage values could not be determined for all lines because of problems with transmission or poor gene expression.

Figure 10. Total distribution of En positions from all primary transpositions and deviant ear progenies from three autonomous source alleles.


NUMBER
55
50
45
40
35
30
25
20
15
10
5
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

En insertion was not random. More transposed *En*'s were mapped in the regions 2-12 map units distal to the *al* locus and 4-30 map units proximal. Fewer *En*'s were mapped near *al* and at the proximal and distal ends of the range examined. The transposed *En*'s were mapped at many locations within the preferred regions and the insertion of *En* does not appear to be limited to a few specific sites. The observed preference of *En* for some regions of the maize chromosome may be due to either the receptivity of the chromosome in those regions or to the process of *En* insertion.

Comparison of the Distribution of *En* from Three Mutable Allele Sources

Are *En* insertions an intrinsic property of the individual *En*? This was tested by comparing the transpositions arising from three *En* sources. The distribution of transposing *En*'s from the *al-m(pa-pu)* allele (Fig. 11) was similar to the total distribution of all sources as were those from *al-pm* (Fig. 12) and from *al-m(Au)* (Fig. 13). The distribution of *En*'s transposing from *al-m(pa-pu)* was not significantly different from the distribution of *al-pm* in Chi-squared contingency tests (Table 5). Chi-squared contingency comparisons were also made between the *al-pm* and *al-m(Au)* alleles (Table 6). No significant differences were found between these two sources. In all three sources, more *En*'s were mapped in the regions 2-12 map units distal to *al* and 4-20 map units proximal. Approximately the same percentage of lines were confirmed to have linkage values significantly different from those of the parent line in all three sources although, within the same source, some lines gave rise to more

Figure 11. Distribution of linkage positions of transposed *En*'s originating from the autonomous *al-m(pa-pu)* allele.

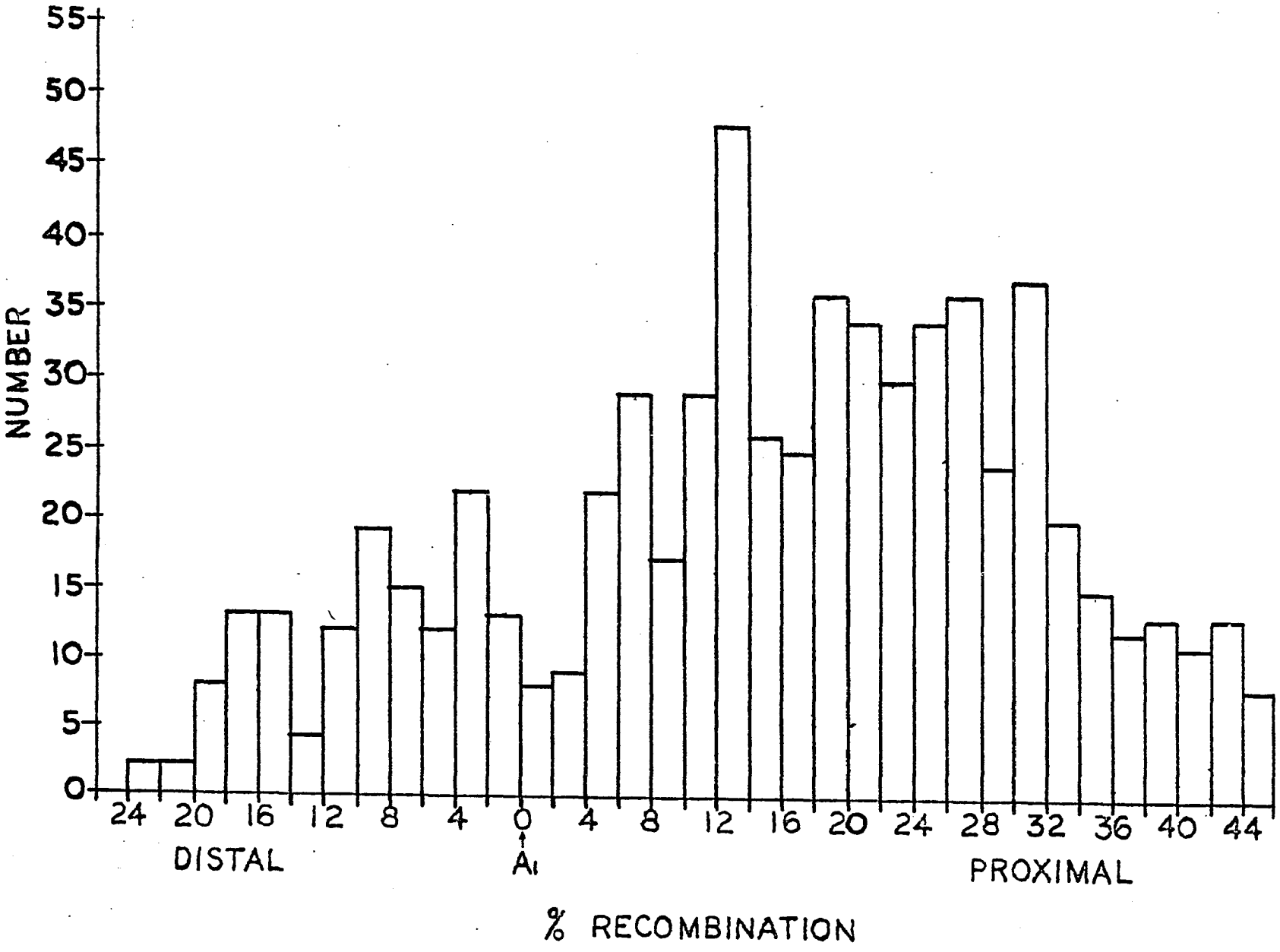

NUMBER
55
50
45
40
35
30
25
20
15
10
5
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

Figure 12. Distribution of the linkage positions of transposed *En*'s originating from the autonomous *al-pm* allele.

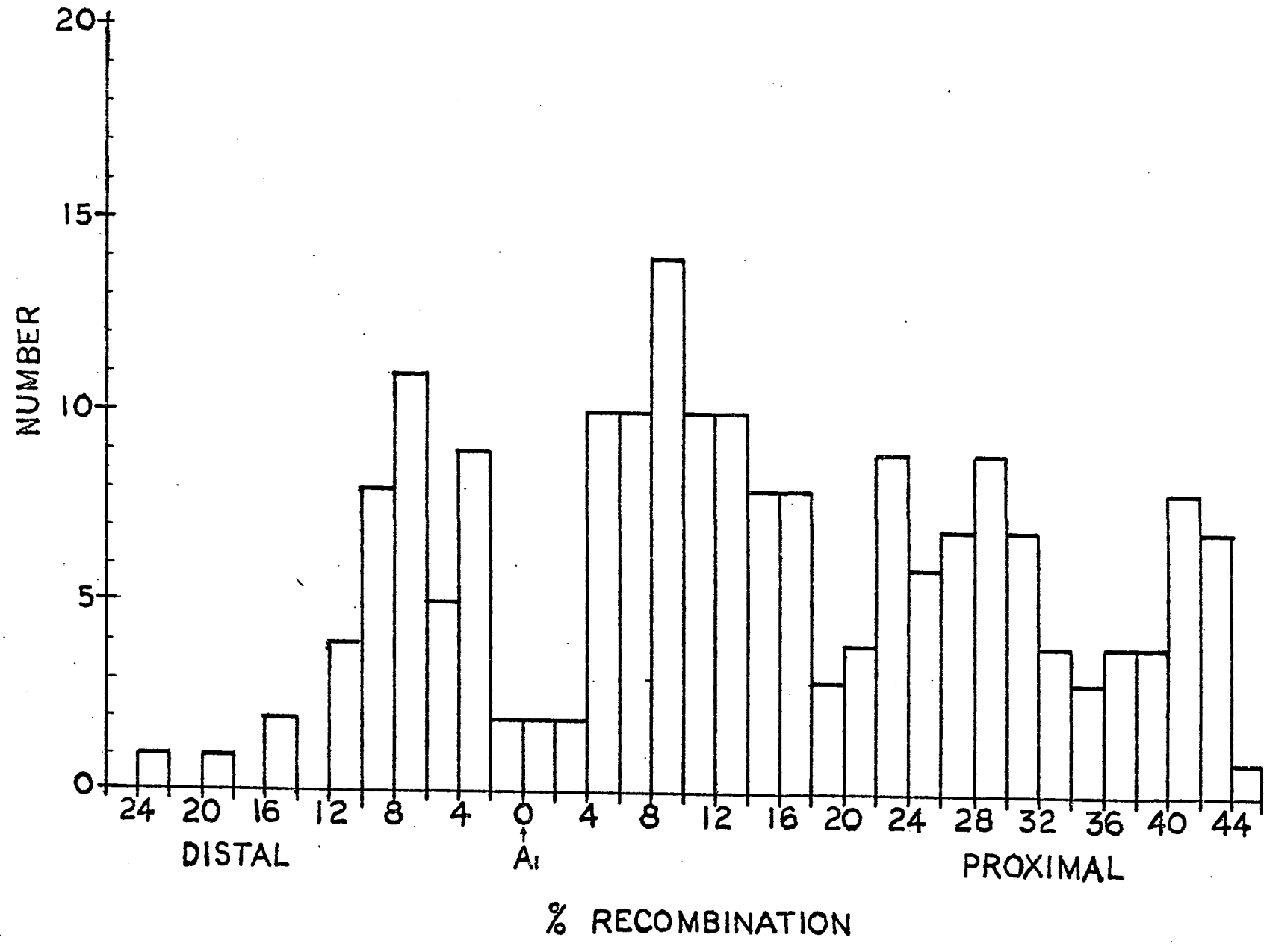

20
15
10
5
0
NUMBER
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

Figure 13. Distribution of linkage positions of transposed *En*'s originating from the *al-m(Au)* allele.

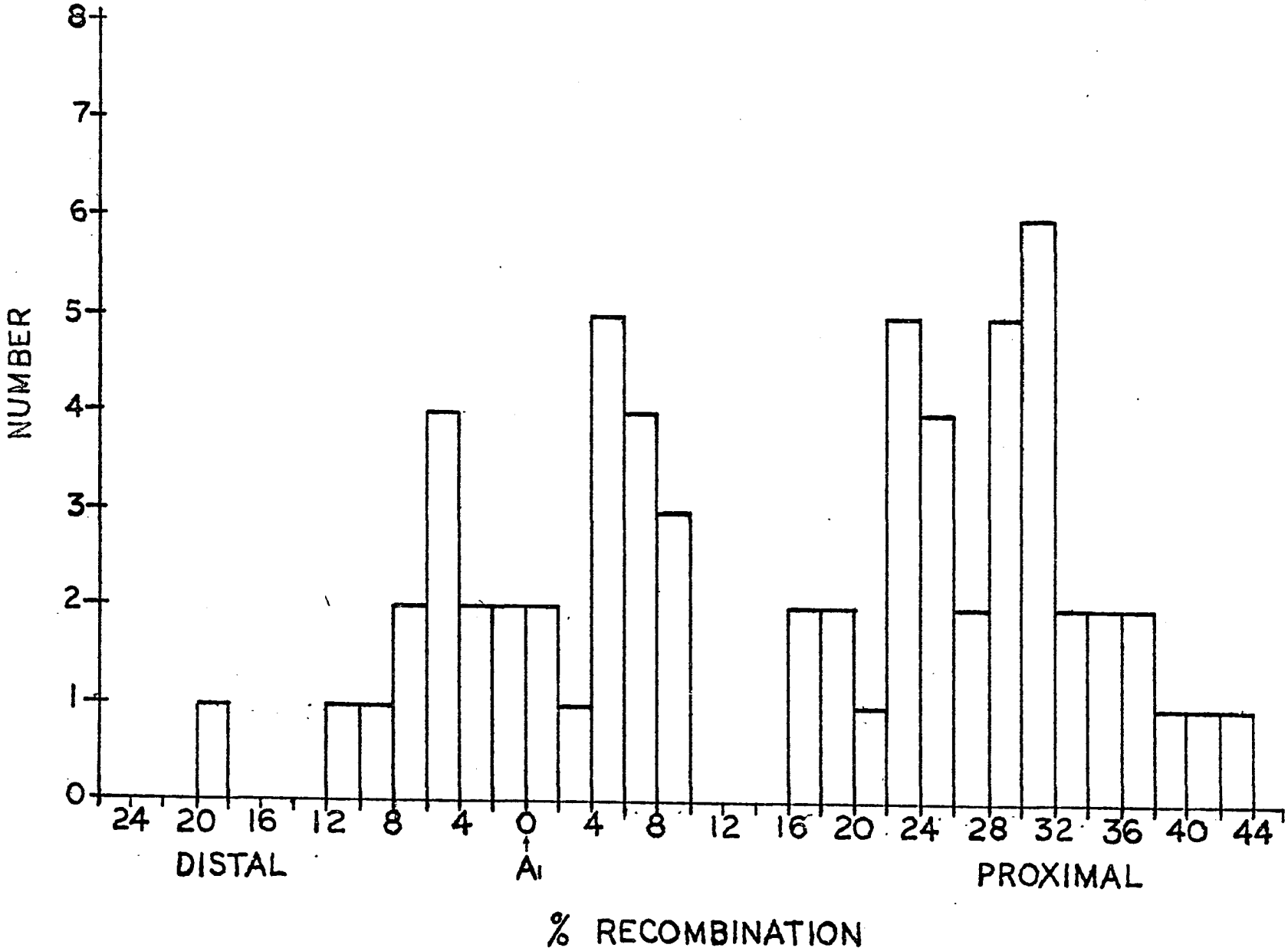

NUMBER
0
1
2
3
4
5
6
7
8
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

Table 5. χ^2 comparison of the distributions of transposed En's from the al-m(pa-pu) and the al-pm alleles

% Recombination	Observed al-m(pa-pu)	Observed al-pm	Observed Total	Expected al-m(pa-pu)	Expected al-pm	Chi-squared values	
24-22	2	1	3	2.33	.67	.05	.16
22-20	2	0	2	1.55	.45	.13	.45
20-18	8	1	9	6.99	2.01	.15	.51
18-16	13	0	13	10.10	2.90	.83	2.90
16-14	13	2	15	11.65	3.35	.16	.54
14-12	4	0	4	3.11	.89	.25	.89
12-10	12	4	16	12.43	3.57	.01	.05
10-8	19	8	27	20.97	6.03	.19	.64
8-6	15	11	26	20.19	5.81	1.33	4.64
6-4	12	5	17	13.20	3.80	.11	.38
4-2	22	9	31	24.08	6.92	.18	.62
2-0	13	2	15	11.65	3.35	.16	.54
0-2	8	2	10	7.77	2.23	.01	.02
2-4	9	2	11	8.54	2.46	.02	.09
4-6	22	10	32	24.85	7.15	.33	1.14
6-8	29	10	39	30.29	8.71	.05	.19
8-10	17	14	31	24.08	6.92	2.08	7.24
10-12	29	10	39	30.29	8.71	.05	.19
12-14	48	10	58	45.04	12.96	.19	.67
14-16	26	8	34	26.41	7.59	.01	.02
16-18	25	8	33	25.65	7.37	.02	.05
18-20	36	3	39	30.29	8.71	1.08	3.74
20-22	34	4	38	29.51	8.49	.68	2.37
24-24	30	9	39	30.29	8.71	.00	.01
24-26	34	6	40	31.06	8.94	.28	.97
26-28	36	7	43	33.39	9.61	.20	.71
28-30	24	9	33	25.63	7.37	.10	.36
30-32	37	7	44	34.17	9.83	.23	.81
32-34	20	4	24	18.64	5.36	.10	.34
34-36	15	3	18	13.98	4.02	.07	.26
36-38	12	4	16	12.43	3.57	.01	.05
38-40	13	4	17	13.20	3.80	.00	.01
40-42	11	8	19	14.76	4.24	.96	3.33
42-44	13	7	20	15.53	4.47	.41	1.43
44-46	8	1	9	6.99	2.01	.15	.51
	671	193	864				

χ^2 = 46.63; DOF = 33
NS at 5% level of significance

Table 6. χ^2 comparison of the distribution of transposed En's from the a-pm and the al-m(Au) (reverse dense) alleles

% Recombination	Observed al-pm	Observed al-m(Au)	Observed Total	Expected al-pm	Expected al-m(Au)	Chi-squared values	
24-22	1	1	5	3.75	1.25		
22-20	0	1	5				
20-18	1	1	5	3.75	1.25	.02	.05
18-16	0	1	5				
16-14	2	1	5				
14-12	0	1	5				
12-10	4	1	5	3.75	1.25	.02	.05
10-8	8	1	9	6.76	2.24	.23	.69
8-6	11	2	13	9.76	3.24	.16	.47
6-4	5	4	9	6.76	2.24	.46	1.38
4-2	9	2	11	8.26	2.74	.07	.20
2-0	2	2	4	3.00	1.00	.33	1.00
0-2	2	2	4	3.00	1.00	.33	1.00
2-4	2	1	3	2.25	.75	.03	.08
4-6	10	5	15	11.26	3.74	.14	.42
6-8	10	4	14	10.51	3.49	.02	.07
8-10	14	3	17	12.77	4.23	.12	.36
10-12	10		10	7.51	2.49	.83	2.49
12-14	10		10	7.51	2.49	.83	2.49
14-16	8		8	6.01	1.99	.66	1.99
16-18	8	2	10	7.51	2.49	.03	.10
18-20	3	2	5	3.75	1.25	.15	.45
20-22	4	1	5	3.75	1.25	.02	.05
22-24	9	5	14	10.51	3.49	.22	.65
24-26	6	4	10	7.51	2.49	.30	.92
26-28	7	2	9	6.76	2.24	.01	.03
28-30	9	5	14	10.51	3.49	.22	.65
30-32	7	6	13	9.76	3.24	.78	2.35
32-34	4	2	6	4.51	1.49	.06	.17
34-36	3	2	5	3.75	1.25	.15	.45
36.38	4	2	6	4.51	1.49	.06	.17
38-40	4	1	5	3.75	1.25	.02	.05
40-42	8	1	9	6.76	2.24	.23	.69
42-44	7	1	8	6.76	2.24	.23	.69
44-46	1		1				
	193	64	257				

χ^2 = 26.67; DOF = 29
NS at 5% level of significance

confirmed linkage changes than other lines. Since no differences were found in the distributions of transposing En's from the three sources, they were combined in the rest of the analysis.

Relation of Sites of Excision and Sites of Insertion

Are the distributions of excision and insertion sites dependent on the process of DNA replication in the maize chromosome? If En is triggered to transpose by the replication of its insertion site and it transposes into unreplicated regions of the chromosome, as proposed by the Greenblatt and Brink models, transposing En's would be expected to insert into the later replicating regions of the chromosome. En's in these later replicating regions would be expected to give rise to fewer confirmed transpositions since only the still unreplicated regions of the chromosome would be available for insertion. If transposition is related to replication,differences between the distribution along the chromosome of En's giving rise to transpositions and the distribution of the insertion sites of the transposing En's may be seen.

Linkage sites of parental lines that gave rise to confirmed linkage changes were plotted (Fig. 14). In each of these cases the En was lost at the parental site and had reappeared at another linked or independent site in a progeny line. An excision of En from the parental site is assumed to have occurred. The frequency distribution of linkage sites of the newly transposed En's is plotted in Fig. 15. En is assumed to have inserted at these positions. The two distributions were not

Figure 14. Linkage sites of parental lines giving rise to confirmed *En* transpositions.

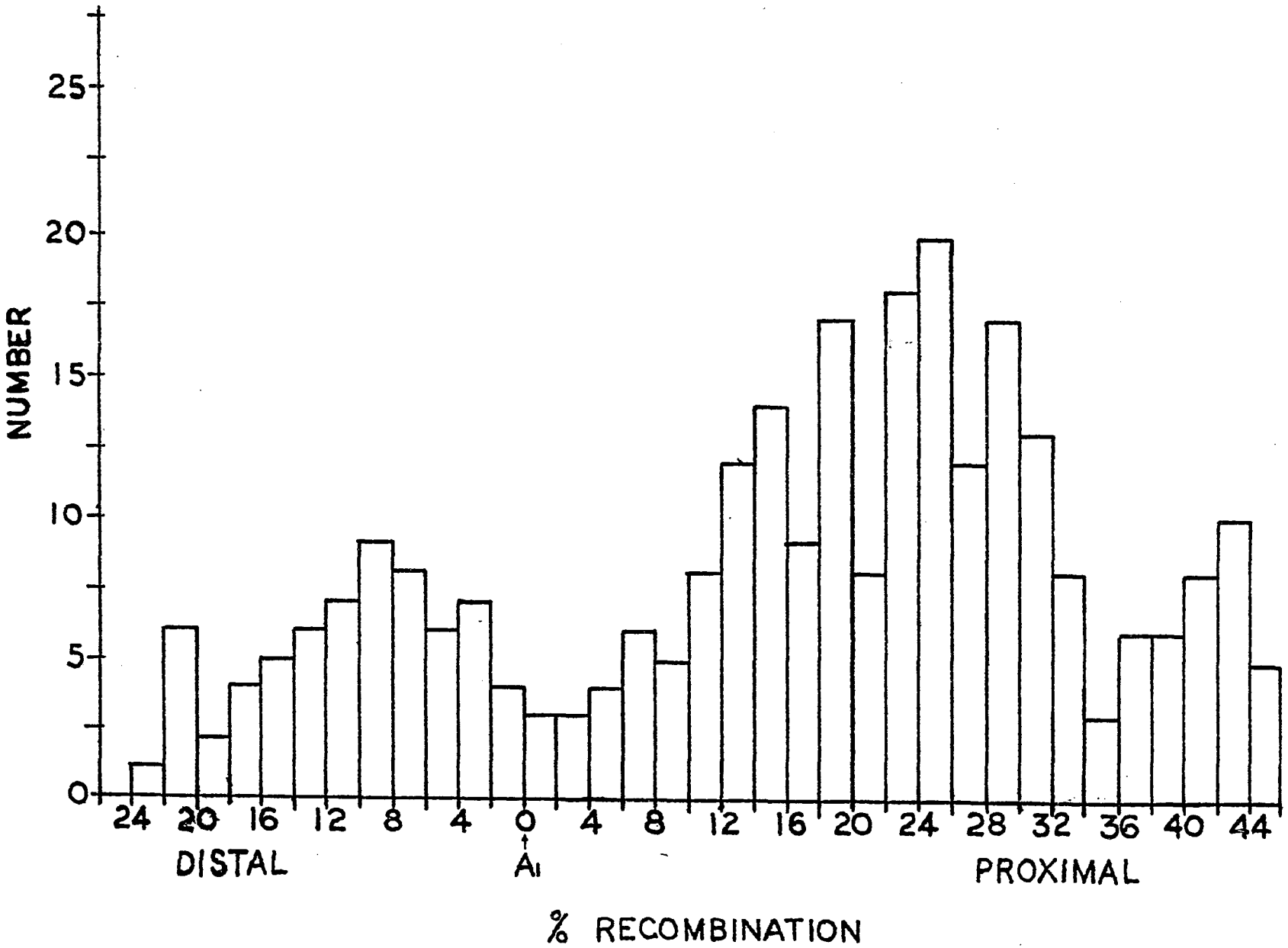
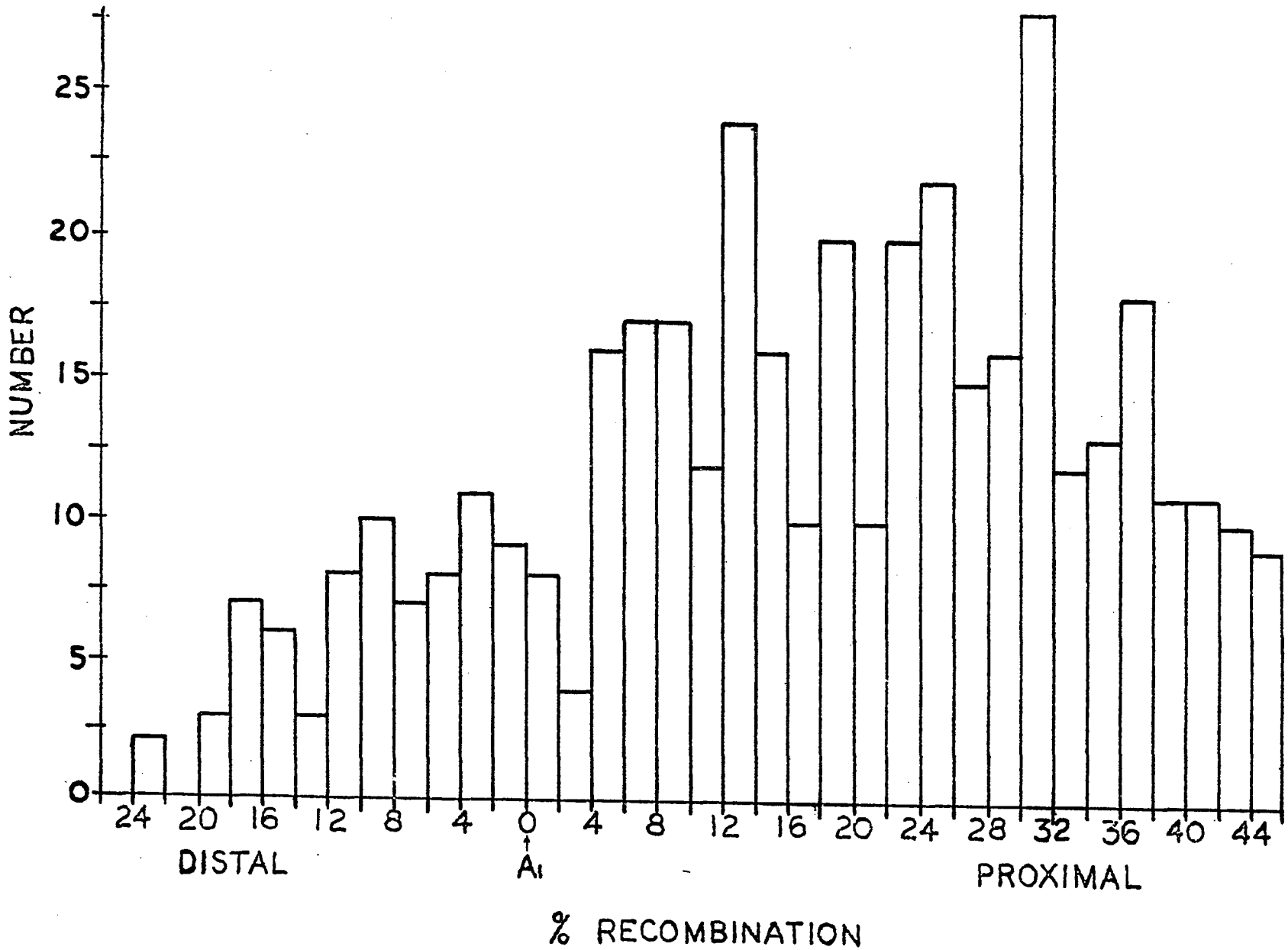

NUMBER
25
20
15
10
5
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

Figure 15. Linkage positions of newly confirmed primary and secondary transpositions.


NUMBER
25
20
15
10
5
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

significantly different in Chi-square contingency tests (Table 7). Those chromosomal regions that were most active in excision were also most receptive to En insertion.

Relation Between Primary and Secondary Sites of Insertion

Do the distributions of primary and secondary transpositions of En away from an autonomous mutable allele depend on the process of DNA replication? The primary positions are the linkage sites that the En assumes when it first transposes from its autonomous position at al. The secondary sites are the linkage positions that En assumes when it transposes from the primary sites. If En prefers to insert into unreplicated DNA the region available for insertion should become smaller with each transposition since only those areas of the chromosome replicating later than the primary site would be receptive to En insertion. If this is the case, the distributions of secondary transposition sites should be more restricted than the distribution of primary sites.

Both primary and secondary sites of En insertion were determined by the method outlined in the Materials and Methods section. Chi-square contingency tests indicated no significant differences in the distributions of the primary and secondary sites arising from the al-m(pa-pu) allele (Table 8).

To determine if En was as active in excision at the secondary sites the numbers of deviant ears identified among lines with En at the primary and at the secondary sites were compared in 2x2 Chi-square contingency tests (Table 9).

Table 7. χ^2 comparison of distributions of linkage sites giving rise to *En* transpositions and linkage sites of newly transposed *En*'s

	Observed			Expected			
% Recombination	Source of transposing *En*	Linkage site of newly transposed *En*	Total	Source of transposing *En*'s	Linkage site of newly transposed *En*'s	Chi-square values	
24-22	1	2	3	1.22	1.78	.04	.03
22-20	6	0	6	2.42	3.58	5.30	3.58
20-18	2	3	5	2.02	2.98	.00	.00
18-16	4	7	11	4.44	6.56	.04	.03
16-14	5	6	11	4.44	6.56	.07	.05
14-12	6	3	9	3.64	1.53	1.53	1.04
12-10	7	8	15	6.06	8.94	.15	.10
10-8	9	10	19	7.68	11.32	.23	.15
8-6	8	7	15	6.06	8.94	.62	.42
6-4	6	8	14	5.66	8.34	.02	.01
4-2	7	11	18	7.27	10.73	.01	.01
2-0	4	9	13	5.25	7.75	.30	.20
0-2	3	8	11	4.44	6.56	.47	.32
2-4	3	4	7	2.83	4.17	.01	.01
4-6	4	16	20	8.08	11.92	2.06	1.40
6-8	6	17	23	9.29	13.71	1.17	.79
8-10	5	17	22	8.89	13.11	1.70	1.15
10-12	8	12	20	8.08	11.92	.00	.00
12-14	12	24	36	14.55	21.45	.45	.30
14-16	14	16	30	12.12	17.88	.29	.20
16-18	9	10	19	7.68	11.32	.23	.15
18-20	17	20	37	14.95	22.05	.28	.19
20-22	8	10	18	7.27	10.73	.46	.31
24-26	18	22	42	16.97	25.03	.54	.37
26-28	12	15	27	10.91	16.09	.11	.07
28-30	17	16	33	13.33	19.67	1.01	.68
30-32	13	28	41	16.57	24.43	.77	.52
32-34	8	12	20	8.08	11.92	.00	.00
34-36	3	13	16	6.47	9.53	1.86	1.26
36-38	6	18	24	9.70	14.30	1.41	.96
38-40	6	11	17	6.87	10.13	.11	.07
40-42	8	11	19	7.68	11.32	.01	.01
42-44	10	10	20	8.08	11.92	.46	.31
44-46	5	9	14	5.66	8.34	.08	.05
	280	413	693				

χ^2 = 36.65 NS at 5% level of significance
DOF-34

Table 8. χ^2 comparison of primary and secondary linkage sites from the al-m(pa-pu) locus

	Observed			Expected			
	1st	2nd		1st	2nd		
12-10	2	1	3	1.55	1.45	.13	.14
10-8	1	0	1				
8-6	0	1	1				
6-4	1	1	2				
4-2	0	0	0				
2-0	0	0	1	3.61	3.39	.04	.04
0-2	1	0	1				
2-4	0	1	1				
4-6	0	0	0				
6-8	1	0	1				
8-10	2	0	2	1.03	.97	1.36	1.45
10-12	1	0	1	.52	.48		
12-14	2	1	3	1.55	1.45	.13	.14
14-16	6	2	8	4.13	3.87	.85	.90
16-18	4	4	8	4.13	3.87	.00	.00
18-20	6	6	12	6.19	5.81	.00	.01
20-22	7	2	9	4.64	4.36	1.20	1.28
22-24	11	6	17	8.77	8.23	.57	.60
24-26	6	3	9	4.64	4.36	.40	.42
26-28	5	8	13	6.71	6.29	.44	.46
28-30	5	7	12	6.19	5.81	.23	.24
30-32	6	12	18	9.29	8.71	1.17	1.24
32-34	6	4	10	5.16	4.06	.14	.00
34-36	1	4	5	2.58	2.42	.97	1.03
36-38	1	2	3	1.55	1.45	.20	.21
38-40	2	4	6	3.10	2.90	.39	.42
40-42	2	5	7	3.61	3.39	.72	.76
42-44	2	2	4	2.06	1.94	.00	.00
44-46	0	0	0				
	81	76	157				

$\chi^2 = 18.28$
DOF = 18

Table 9. Comparison between number of deviant ears (A) and ears with an independent En (B) between lines with En at a primary site and lines with En at a secondary site

A.

	Observed		Expected	
	No. ears examined	No. deviant	No. ears examined	No. deviant
Primary	875	137	878.69	133.31
Secondary	819	120	815.31	123.69

χ^2 = .24; NS at 5% level of significance.

B.

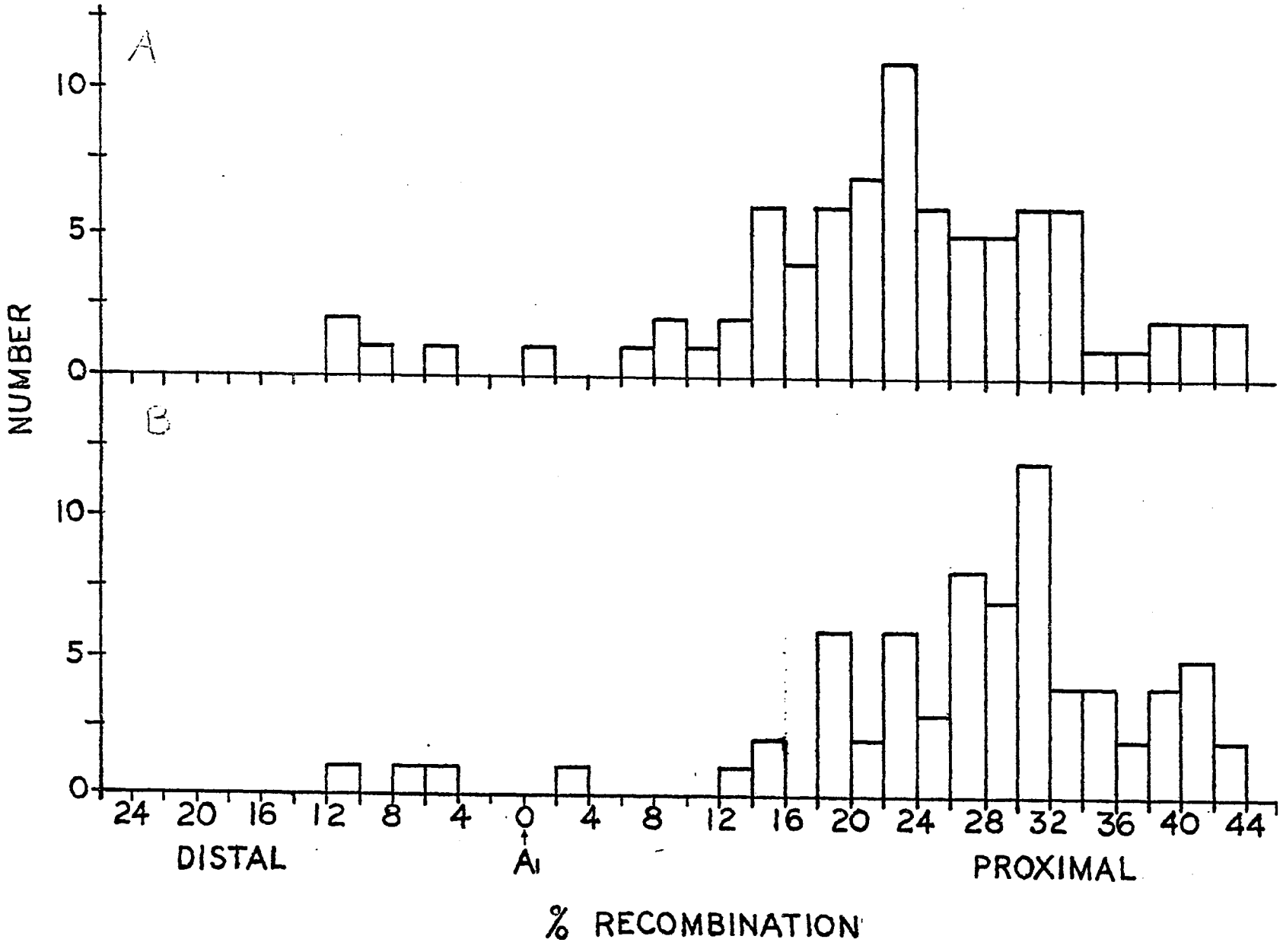
Primary	137	23	134.38	25.62
Secondary	120	26	122.62	23.38

χ^2 = .67; NS at 5% level of significance.

Figure 16. Comparison of primary and secondary sites of insertion of transposing *En*'s

A. Distribution of primary positions of *En* insertion.

B. Distribution of secondary positions of *En* insertions.


A
B
NUMBER
10
5
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
AI
PROXIMAL
% RECOMBINATION

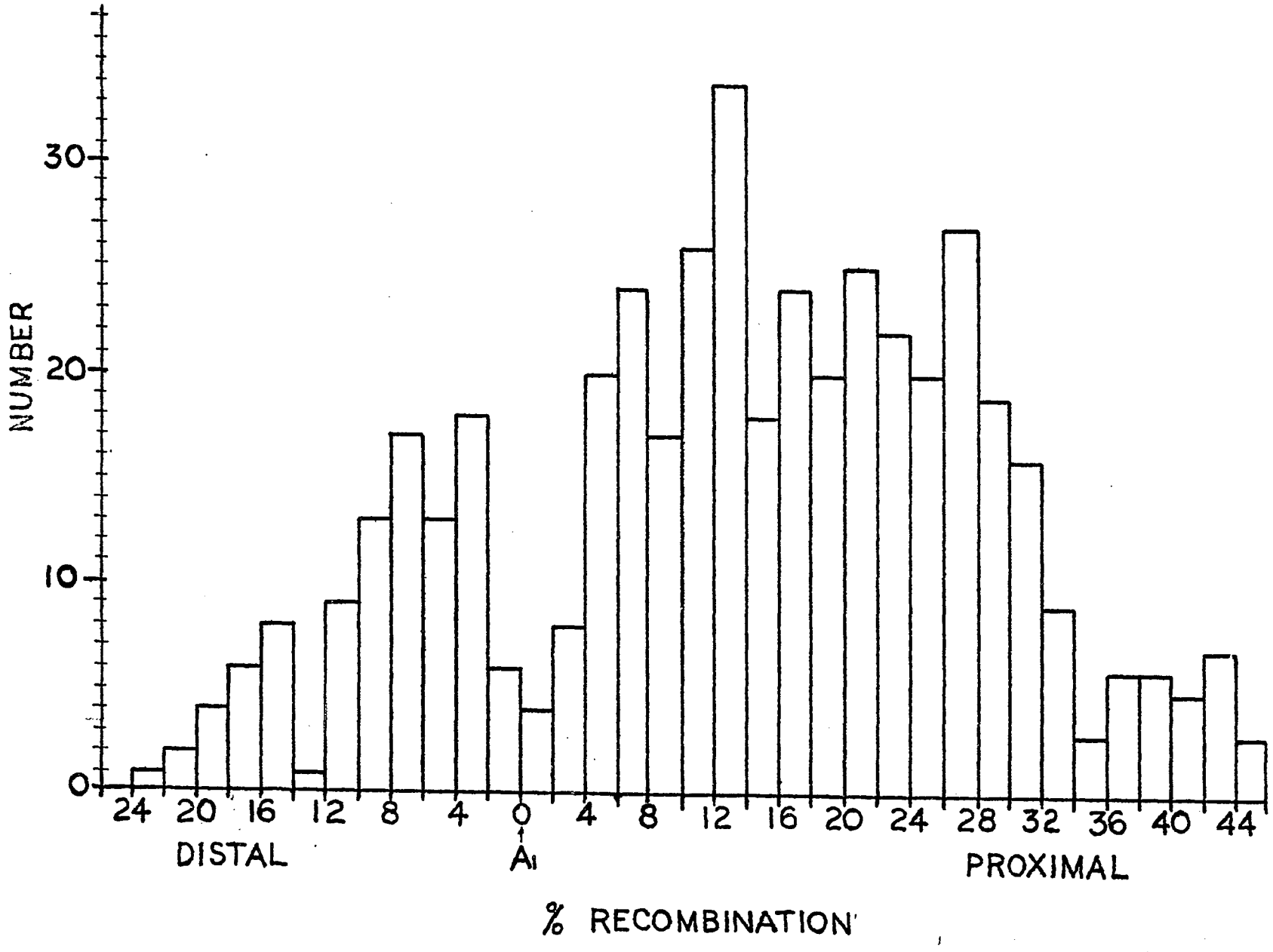
If the receptive region of the chromosome is smaller in the secondary transpositions, it would be expected that En would move to an independent position more often in secondary than in primary transpositions. The number of ears with an independent En arising from lines with En at a primary and at a secondary site were compared in 2x2 Chi-square contingency tests (Table 9).

No significant differences were found in the number of deviant ears or the number of independent En's between the two sets of lines. There was no indication of any differences in the transpositional activity of En in the secondary linkage positions and the number of independent En's seemed to be arising at a constant rate. Since the distributions of primary and secondary sites were the same and there was no more tendency for En to move into an independent position from the secondary sites, it appears that the same regions of the chromosome are available for En insertion in both primary and secondary transpositions.

Distance and Direction of Transposition

In order to determine if En has a tendency to move a certain distance or a specific direction in relation to the centromere, the distance in map units and distal or proximal direction of movement of the confirmed transpositions from the parental site to the newly confirmed progeny site was determined. A frequency diagram of the distance and direction of En transposition is presented in Fig. 17. Few values are mapped between 5 map units proximal and 5 units distal

Figure 17. Distance and direction of En transposition in map units.


NUMBER
30
20
10
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

because transpositions of less than 5 map units could not be detected by changes in linkage values. However, there was a tendency for the En element to move shorter distances and most of the detected transpositions were between 6 and 16 map units away from the parental site. Although there was a tendency for En to move shorter distances, there was no loss of efficiency of insertion in transpositions to sites at greater distances. Further, about 40% of the lines tested had at least one En at an independent position. There was no tendency for En to move in either the proximal or distal direction.

Although differences among the al-m(pa-pu), al-pm, and al-m(Au) sources in the distance and direction of transposition were not tested statistically, they appeared to behave similarly. En's originating at all three alleles showed a tendency to move shorter distances along the chromosome and no preference for movement in either the proximal or distal direction was observed.

Stable and Active Chromosomal Regions

To determine if chromosomal regions differ in the frequency of En transposition, the frequency distribution of linkage sites that gave rise to confirmed transpositions (Fig. 14) was compared to the distribution of lines in which no transpositions were detected (Fig. 18). The two distributions were found to be significantly different at the 5% level of significance in Chi-squared contingency tests (Table 10). Confirmed transpositions arose from parental lines with En's mapped

Figure 18. Linkage sites of parental lines giving rise to a low frequency of confirmed *En* transpositions.

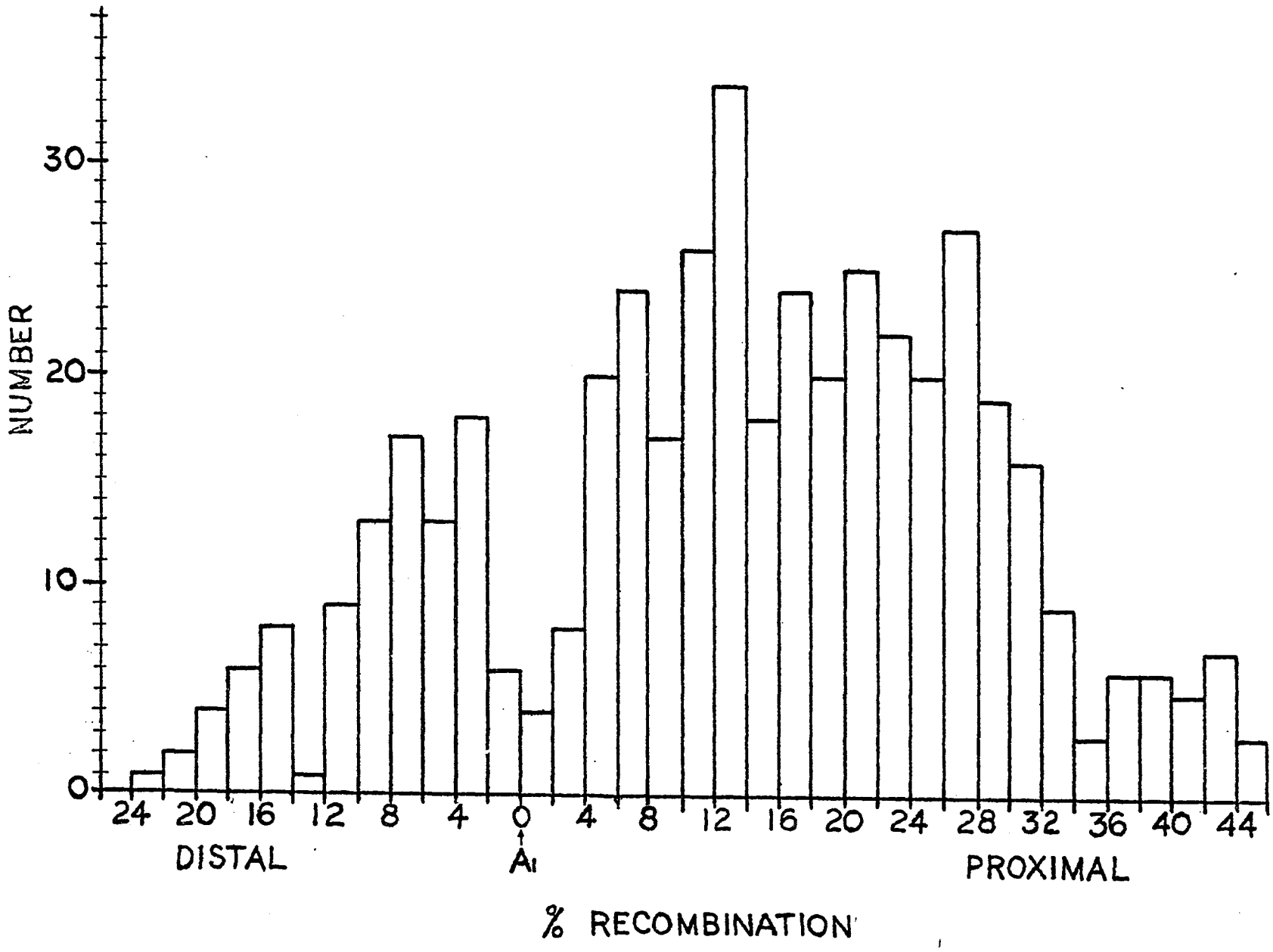

NUMBER
30
20
10
0
24 20 16 12 8 4 0 4 8 12 16 20 24 28 32 36 40 44
DISTAL
A1
PROXIMAL
% RECOMBINATION

Table 10. χ^2 comparison between linkage site giving rise to transpositions and linkage site of non-deviant progeny lines

	Observed			Expected			
	Gave rise to transpositions	Stable		Active	Stable	Chi-square	Values
24-22	1	1	2	.76	1.24	2.74	1.67
				3.78	6.22		
22-20	6	2	8	3.02	4.98		
20-18	2	4	6	2.27	3.73	.03	.02
18-16	4	6	10	3.78	6.22	.01	.01
16-14	5	8	13	4.91	8.09	.00	.00
14-12	6	1	7	2.65	4.35	4.23	2.58
12-10	7	9	16	6.05	9.95	.15	.09
10-8	9	13	22	8.31	13.69	.06	.03
8-6	8	17	25	9.45	15.55	.64	.39
6-4	6	13	19	7.18	11.82	.19	.12
4-2	7	18	25	9.45	15.55	.64	.39
2-0	4	6	10	3.78	6.22	.01	.01
0-2	3	4	7	2.65	4.35	.05	.03
2-4	3	8	11	4.16	6.84	.32	.20
4-6	4	20	24	9.07	14.93	2.83	1.72
6-8	6	24	30	11.34	18.66	2.51	1.53
8-10	5	17	22	8.31	13.69	1.32	.80
10-12	8	26	34	12.85	21.15	1.83	1.11
12-14	12	34	46	17.38	28.62	1.67	1.01
14-16	14	18	32	12.09	19.91	.30	.18
16-18	9	24	33	12.47	20.53	.97	.57
18-20	17	20	37	13.98	23.02	.65	.40
20-22	8	25	33	12.47	20.53	1.60	.97
22-24	18	22	40	15.11	24.89	.55	.34
24-26	20	20	40	15.11	24.89	1.58	.96
26-28	12	27	39	14.74	24.26	.51	.31
28-30	17	19	36	13.60	22.40	.85	.52
30-32	13	16	29	10.96	18.04	.38	.23
32-34	8	9	17	6.42	10.58	.39	.24
34-36	3	3	6	2.27	3.73	.23	.14
36-38	6	6	12	4.53	7.47	.48	.29
38-40	6	6	12	4.53	7.47	.48	.29
40-42	8	5	13	4.91	8.09	1.94	1.18
42-44	10	7	17	6.42	10.58	2.00	1.21
44-46	5	3	8	3.02	4.98	1.30	.79
	280	461	741			33.29	

χ^2 = 53.10; DOF = 33; P<.05 = finding of larger value

in all regions of the length of chromosome surveyed and non-deviant lines also appeared within all regions, but more stable lines mapped closer to the al locus while the actively transposing lines tended to map a little farther away. However, this difference in distribution could have been due to sampling errors because non-deviant ears were not selected to test for stability. Ears that were different in linkage value from the parental line average were selected, but in some cases the progeny linkage values of the deviant ears and the parental line averages were not found to be significantly different. It was assumed that no transpositions had arisen in these parental lines though non-detectable linkage changes may have occurred.

To confirm that the parental lines with no confirmed deviant progeny lines were actually more stable than the lines with confirmed transpositions, Chi-squared contingency tests comparing the number of deviant ears arising in the "stable" and actively transposing lines were compared. The proportion of deviant ears was found to be significantly different in the two sets of lines (Table 11).

Relationship Between Pattern Change and Transposition

The three autonomous sources of En had very distinctive patterns of mutability. The transposed En's gave rise to patterns on the standard tester alleles that had no resemblance to the pattern of the autonomous parental sources. The transposed En's gave a similar array of patterns on the standard lines irrespective of the source allele. Frequency distributions for timing and frequency of mutation for lines from the

Table 11. Comparison of number of deviant ears from stable and actively transposing lines

	Observed		Expected	
Line type	No. ears	No. deviant	No. ears	No. deviant
Active	954	187	1026.88	114.12
Stable	3869	349	3796.12	421.88

$\chi^2 = 65.70$* significant at 1% level of probability.

*From these results, it would appear that lines with no detected transpositions were indeed more stable than those giving rise to confirmed transposition.

a1-m(pa-pu) amd a1-pm sources that had been rated visually against a standard set of kernels were compared using Chi-square contingency tests (Table 12). The distribution of pattern types originating from the two source alleles were not significantly different in either timing or frequency of mutation and the arrays of patterns produced by the En's tranposing from these two sources were similar. These data support the position hypothesis which states that the pattern of mutability generated by the En element is not an inherent property of the element itself but depends on its specific position on the chromosome.

There is a definite association between transpositional events and pattern changes and transpositions can often be identified by the pattern changes produced. Single seeds that appeared to differ from the parental ear were collected and grown. If the ear produced from the single seed showed a confirmed change in pattern a twelve seed sample was saved and the progeny linkage value determined.

Of the 38 lines that showed a confirmed change in pattern, 23 had also undergone a confirmed transposition. Whole ears that differed in pattern from the parental line were also collected and tested for transposition. Of 131 ears that showed a pattern change, 86 also had a confirmed transposition. This is probably an underestimate of the total number of transpositions, however, because linkage changes of less than 5% could not be detected.

Although there was a definite association between transposition and pattern change, no correlation was found between the linkage position

Table 12. χ^2 comparison between distribution of timing of mutation of transposed En from the al-m(pa-pu) and al-pm alleles

Timing	pa-pu	pm			
100	4	0	4	3.53	.46
90	8	0	8	7.07	.93
80	27	0	27	23.86	3.14
70	10	4	14	12.37	1.63
60	36	2	38	33.58	4.42
50	64	14	78	68.92	9.08
40	10	1	11	9.72	1.28
30	6	1	7	6.18	.81
20	1		1	1.77	.23
10	1		1		
	167	22	189		

$\chi^2 = 13.61$; DOF = 6
NS at the 5% level

40	81	9	90	79.52	10.48
35	6	0	6	5.30	.70
30	63	12	75	66.27	8.73
25	4	0	4	3.53	.47
20	13	1	14	12.37	1.63
	167	22	189		

$\chi^2 = 3.21$; DOF = 4
NS at the 5% level

of En and specific pattern produced based on the frequency or timing of mutation (Table 13). No association was found between any particular linkage position and pattern (Figs. 19 and 20). More lines were found to have patterns in the middle range of timing and frequency, but lines with linkage values throughout the linkage region studied could have these patterns. Linkage values could not be determined for most of the lines with low timing and frequency values. In many cases, En's with weak mutation patterns either were not transmitted normally or became inactive. Linkage values from the lines with few, small spots were usually calculated at over 50% when based on either the number of mutable or colored kernels per se. The lack of correlation between pattern and linkage value was not unexpected because a specific linkage value is a very rough standard of the exact En position.

Table 13. General linear models procedure for correlation of timing of mutation and linkage of transposed En

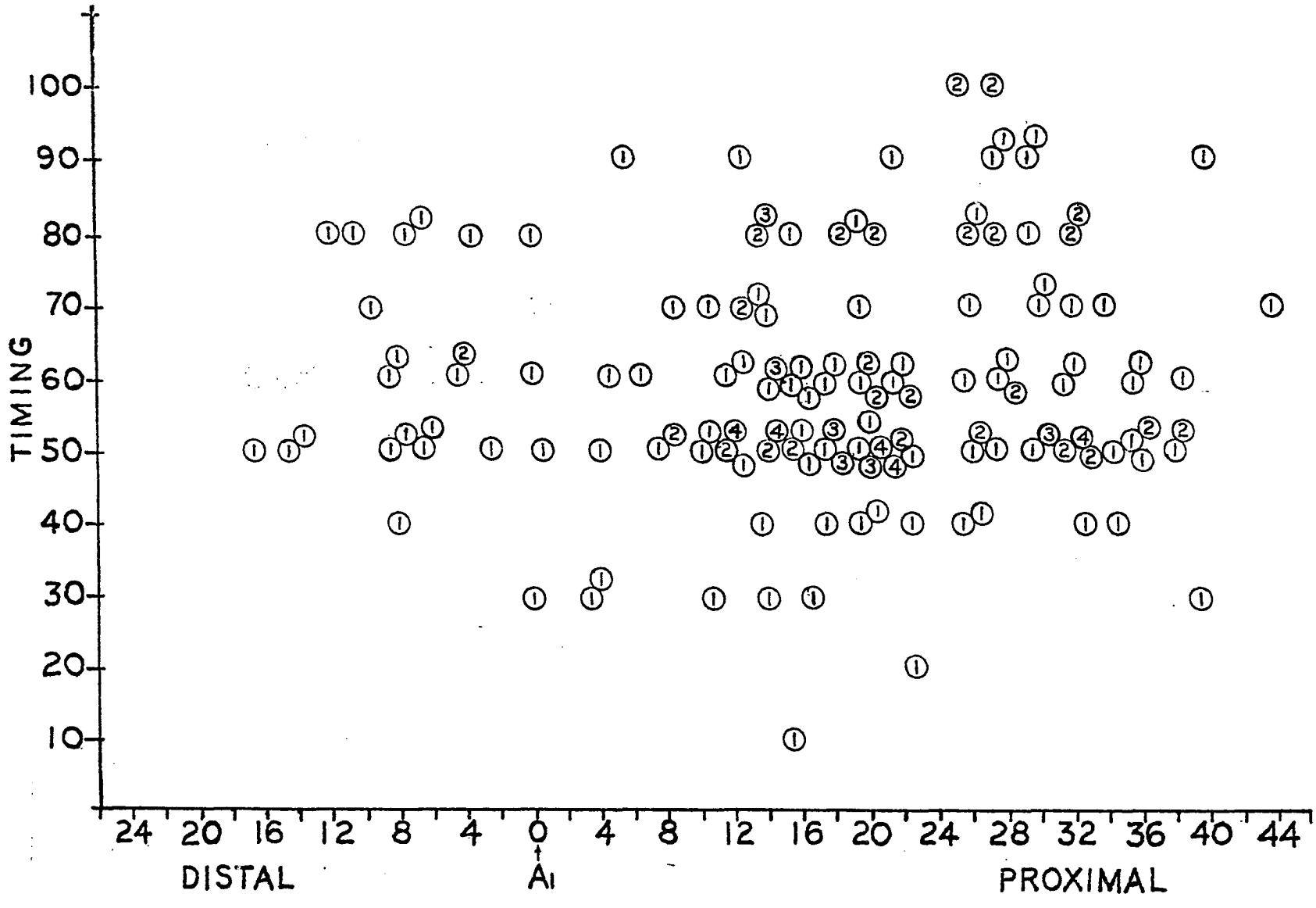
Dependent variable: T

Source	DF	Sum of squares	Mean square	F value	PR F	R-square	C.V.
Model	1	171.76	171.76	0.67	0.41	0.00	27.16
Error	188	47986.80	255.25		Std. Dev.		T Mean
Corrected total	189	48158.55			15.98		58.82

Source	DF	Type 1 SS	F value	PR F	DF	Type IV SS	F value	PR F
CD	1	171.76	0.67	0.41	1	171.76	0.67	0.41

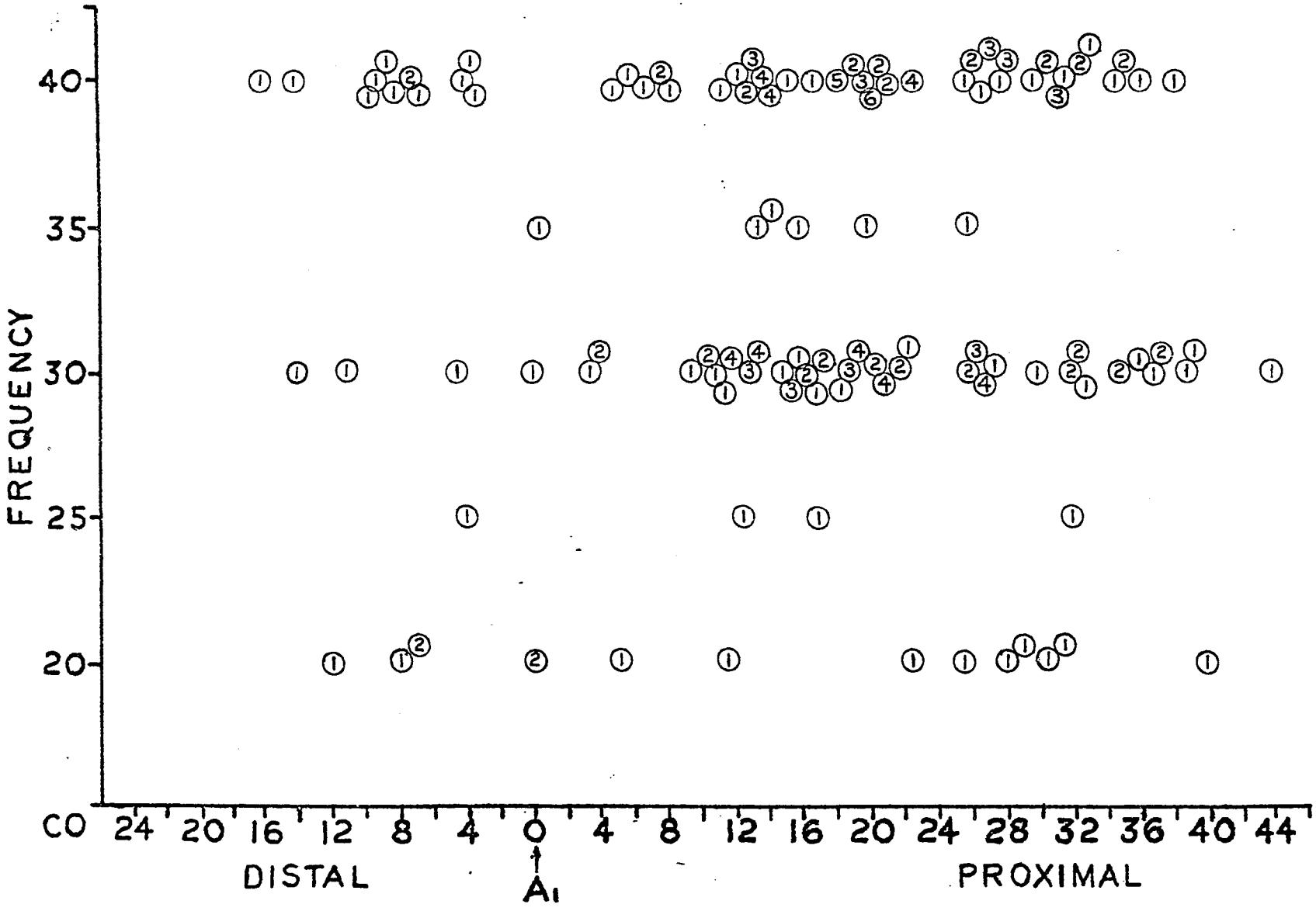
Parameter	Estimate	T for Ho: parameter=0	PR 1T1	Std Error of estimate
Intercept	57.45	28.32	0.00	2.03
CD	0.07	0.82	0.41	0.09

Figure 19. Relationship of timing of En mutants to En position.



RELATION BETWEEN TIMING OF MUTATIONS AND LINKAGE POSITION

Figure 20. Relationship of frequency of En mutation to En position.



RELATION BETWEEN FREQUENCY OF MUTATION AND LINKAGE POSITION

DISCUSSION

Results of this study support several features of En transposition: 1) there are preferred regions of En insertion within the long arm of chromosome 3, 2) the En's originating from three different autonomous, mutable alleles show the same regional preferences, 3) the En's in some regions of the chromosome are active in excision and insertion while En's in other regions are less likely to transpose, 4) the distribution of En along the assayed part of chromosome 3L does not change in subsequent generations of transposition, and 5) there is a propensity for En to move shorter distances. 6) While En transposition was often associated with changes in state, there is no correlation or association between the state of En and its linkage position.

Regional Preference of En

The sites of En insertion can be affected by the insertion activity of the En element and by the receptivity of specific regions of the maize chromosome.

In some of the bacterial transposons, the enzymes responsible for insertion cleave the host DNA at specific base pair sequences (Landy and Ross, 1977), while in others no specific sequence is required for insertion (Bukhari, 1976). If En has a specific base pair sequence required for insertion it might show a preference for regions where there is a higher concentration of those sequences. However, if En has a specific base pair sequence requirement for insertion, it would have to be a very short or highly repetitive sequence. Numerous linkage sites of En have

been identified within the segment of 3L considered in this study. En's have been found within every target locus considered (Peterson, 1978). The receptor elements of the En system have been mapped at two separate locations within the wx locus (Nelson, 1968). There is also evidence for multiple insertion sites of the Mp element within the R locus (Brink and Williams, 1973).

The regional preference of En can also be affected by the receptivity of the maize DNA in those regions. The DNA receptivity could be caused by gene activity in those regions, the replication of the chromosomal region, or by some general feature of the chromosomal structure.

In both the Greenblatt and the Brink models, Mp transposition is associated with replication. Their observations suggested that there is a preference for insertion into unreplicated segments of DNA. If En also has a preference for insertion into unreplicated regions of DNA, it would appear that more En insertion would occur in the later replicating regions. This raises questions as to whether the regions of En preference identified in this study are related to regions of later replicating DNA in maize. To answer this question, the process of DNA replication should be examined.

Effect of DNA Replication on Distribution of Transposing En's

DNA replication in eukaryotes begins at multiple initiation sites (Wimber, 1961). The segments between initiation sites which are replicated as a unit are referred to as replicons. Although no specific initiation

and termination sequences are known in eukaryotes, there is evidence for their presence both in eukaryotes and prokaryotes. In bacteria, there is one initiation site in the circular chromosome and the specific sequence has been identified in some species (Wake, 1974). While there are multiple initiation sites in eukaryotes, the process seems to be under a similar type of control.

In Drosophila, a mutant has been identified at the white locus that generates many small germinal deletions restricted to two specific cytological bands (Gethman, 1972). This mutant behaves as if it contains a misplaced replication terminator signal. It seems that initiation and termination of replication are not random processes but they are both under genetic control.

Replicon size has been estimated to average about 24 μm in Crepis capillaris which would be about 72,000 base pairs in length (Van't Hof and Bjerknes, 1979). Replication in higher plants is relatively slow and occurs at about 9.1 μm/hr at 23° C. There would be ample time for a controlling element to move within a single replicon while the replicon is actively replicating, but because a replicon is relatively small the transposition would not necessarily be detected by a change in linkage value. A transposon would be free to move back and forth between synchronously replicating replicons and the transpositions could be detected by linkage changes if the replicons were far enough apart. However, if insertion is restricted to unreplicated regions the regions available for insertion would become smaller with each transposition.

If transposition is triggered by the process of replication at the original site of controlling element insertion and if the controlling element moves into unreplicated regions, only the regions replicating later than the site of excision would be available for reinsertion. The available regions would shrink with each subsequent transposition and differences of the insertion regions should be detected between early and later transpositions.

No difference between the regions of En insertion in primary or secondary transpositions was found in this study, suggesting that the regions available for insertion did not become more restricted in the two generations of transposition studied. En's were no more likely to move to independent positions in the secondary transpositions although more En's would be expected to move out of the segment surveyed if fewer insertion sites were available. Of course, DNA replication is relatively slow in plant species and it may take several generations of transposition before a more restricted region of insertion becomes evident.

Replicons replicate asynchronously along the eukaryotic chromosome and the actively replicating replicons tend to cluster (Wolstenholme, 1973). By treating Drosophila eggs with radioactively labelled DNA precursors early and late replicating regions could be identified among the Drosophila chromosomes. Double-stranded bubbles appeared along the chromosome where clusters of early replicating replicon joined together.

Studies using radioactively labelled DNA precursors have also been conducted in a few plant species. In Crepis capillaris DNA

replication was found to begin at the distal ends of the chromosome and to move towards the centromere (Taylor, 1958). In Tradescantia, however, replication begins at sites equally distributed along the chromosome but proceeds more rapidly in the centromeric regions so that the distal regions continue to replicate when replication is complete in other regions of the chromosome (Wimber, 1961). The later replicating regions in Tradescantia are not related to known segments of heterochromatin.

If the pattern of DNA replication in maize is similar to either of these species and if En inserts preferentially into late replicating regions, En would be expected to move into either the distal or proximal regions of the chromosome. However, En does not accumulate in either the proximal or distal region of the chromosomal segment examined in this study. No tendency was noted for En to move to either the distal or proximal direction as would be expected if replication proceeded in either direction and En was moving into unreplicated regions.

Based on the information available on DNA replication in higher plants and on the number of generations of transposition studied, the process of DNA replication does not seem to affect the movement of transposing En's.

From the information available on DNA replication in higher plants, it appears that En transposition is not related to transcription in maize DNA and there appears to be no relation of En transposition to DNA translation and En transposition can occur out of active or inactive genes. This postulate is supported by the following type of experiment (Peterson, unpublished).

The A2 gene acts earlier in the anthocyanin pathway than A1. When the anthocyanin pathway is blocked at A2, the A1 gene activity should be inhibited. In crosses of a2/a2 a1/a1-m(pa-pu) x A2/A2 a1/a1, colorless kernels appear along with the expected full-colored and mutable types. These kernels arise from an excision of the a1-m(pa-pu) allele in the a2/a2 A1/a1-m(pa-pu) parent while the alleles at a1 were quiescent. If transposition of En occurred only when the A1 gene were actively translating no colorless kernels would have been observed.

A possible model of En transposition is found in the TN1 bacterial transposon (Weinstock et al., 1979). Like En, TN1 shows regional preferences along the bacterial DNA, but within these regions there are numerous insertion sites and TN1 has no apparent sequence requirement for insertion. The regions for preferred TN1 insertion are known to be rich in AT base pairs. DNA sequences rich in A and T tend to dissociate at a lower temperature than the G and C rich sequences (Marmur and Doty, 1962). It may be that the A and T rich sequences dissociate more often or unwind within the cell and are available for controlling element insertion more often than regions relatively poor in A and T. The IS2 element seems to be more active in the formation of duplications with new promotor activity when located in an AT rich region of its host (Sommer et al., 1979) and it may be that excision is also more likely to occur in these regions.

Effect of Proximity of the Donor to the Receptor Regions on the Distribution of Transposing En's

A tendency was found to move shorter distances along the chromosome. More transpositions occurred to sites 5-20 map units from the site of excision, but movement was not limited to the regions closer to the point of excision and En showed no loss of efficiency in insertion when transposition occurred to more distant sites. In 40% of the lines tested at least one En was present at an independent site. This is an underestimate since deviant ears that appeared to be independent were not always tested in progeny lines. In an earlier study, 75% of the En's transposing from the a-m(pa-pu) allele were located at an independent site (Peterson, 1970a).

Similar results were found in a study of reconstitution of the P-vv allele (Orton, 1966). The percent reconstitution of P-rr Mp was found to be in negative correlation to the map distance of the transposed Mp element. However, Mp has a lesser tendency than En to move into an independent position.

En probably has an identifiable tendency to move shorter distances, but map distances do not necessarily indicate physical distances, especially during the vegetative stage of the cell cycle when the maize chromosome are randomly coiled and overlapped.

Stability of Some Regions of Enhancer Insertion

The stability of En's at some sites along the chromosome does not seem to be due to a change in the excision capability of the En itself.

There was no association between the pattern type and those regions of the chromosome associated with stability. If the M gene activity (Fig. IV) were weaker at those positions, the pattern of mutability would also have become weak with few and small color sectors. The inefficiency of excision in the stable regions must be due to the position of the Enhancer element since the excision of the Inhibitor element could produce any pattern while En was present at a stable site.

The stability of En at sites at which it was non-active in transposition, like the En at actively transposing sites, is probably independent of DNA replication or transcriptional activity. If DNA replication triggers transposition, parental lines with En at any site should give rise to equal numbers of deviant ears although En's moving from late replicating regions would find fewer available insertion sites and would tend to move to independent sites or become lost. However, the non-transposing En's were found to produce fewer deviant ears as if fewer excisions were actually occurring.

It is known that the process of excision in bacterial transposons is not always precise (Peterson et al., 1979). The deletion formation process depends on the presence of the insertion element, but deletion formation can occur without its removal (Ahmed and Scraba, 1978). Deletion formation is independent of the IS excision process because the two processes show a differential temperature response (Reif and Saedler, 1975) and a gene, independent of rec A, has been mapped in E. coli which controls IS1 mediated deletion formation (Nevers et al., 1976).

Many of the deletions in bacteria mediated by insertion elements result in partially active or null mutants. In some cases, deletions or duplications can give rise to genes that still appear to be completely active. Sometimes the deleted base pairs can become incorporated into the transposon itself. Deletions can also occur during transposition of the maize controlling elements resulting in the same types of mutants as in the bacterial systems. It may be that the non-transposing En's contain base pairs added from the maize chromosome within their insertion sequences that can interfere with the excision process. Insertion must occur normally, but the added base pair sequences may inhibit the activity of the enzyme responsible for excisions. If En requires a short base pair sequence for insertion, the added DNA and of the normal En's would be different.

An alternative explanation is found in the TN1 bacterial transposon system (Weinstock et al., 1979). If the regions of the chromosome where En does actively insert and excise are rich in A and T base pairs as in the regions of Tn1 insertion, it is possible that the stable regions are relatively rich in G and C. If excision is a function of the enzyme encoded in the M gene as proposed by the Nevers-Saedler model (Fig. 4), it may be that tighter coiling in these regions makes them less excessible to the M gene product.

None of the possible explanations for differences in En insertion and excision activity in some chromosomal regions can be completely ruled out. However, the Tn1 model seems to provide the most plausible explanation.

Relation Between Change in Pattern and Change in Linkage Position

The state of En is not an inherent property of the controlling element itself and changes in pattern are definitely related to change in position (Williams and Brink, 1973; Peterson, 1970a; Peterson, (1976b). Although the autonomous sources considered in this study had very different patterns when En was associated with the al locus, they gave an identical distribution of states on a standard tester after transposition. These results confirm an earlier study that showed no correlation between the patterns produced by En before and after transposition (Peterson, 1970a). However, no correlation was found between the linkage position of En and the timing or frequency of mutation and no association was found between any particular pattern and any specific location of En. This result is not unexpected in view of two contingent factors. There are apparently a large number of insertion sites and the exact insertion sites could not be estimated precisely under the limits imposed by linkage.

The fact that no correlation can be found between pattern and linkage position indicates that diffusion of the mutator (M) product is not a limiting factor in determining En state. Low frequency patterns are not located farther from al nor are high frequency patterns closer.

The position hypothesis (Peterson, 1976b) is supported by the fact that distribution of the patterns generated by En's from the al-pm and al-m(pa-pu) sources are identical.

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